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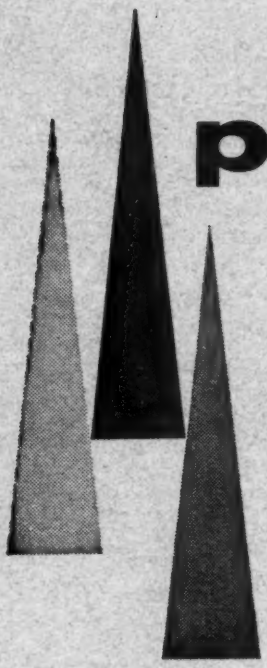

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Studies on the lucerne saponins. I. The isolation of saponins. W. A. Lourens and M. B. O'Donovan (Faculty of Agric., Univ. Natal, Pietermaritzburg). *S. African J. Agric. Science*, 1961, **4**, 151.

The isolation and structure of gafrinin, a sesquiterpenoid lactone from *Geigeria africana*. J. P. de Villiers (Nat. Chem. Res. Lab., Pretoria). *J. Chem. Soc.* 1961, 2049-2051.

Alkaloids of the amaryllidaceae. Krigenamine: A new alkaloid from *nerine krigeii*. P. W. Jeffs and F. L. Warren (Univ. Natal, Pietermaritzburg). *Chem. and Ind. (London)*, 1961, 468.

Use of cation exchange resins in organic reactions .I. The von Pechmann reaction. E. V. O. John and S. S. Israelstam (Univ. Witwatersrand, Johannesburg). *J. Org. Chem.*, 1961, **26**, 240-242.

Some glycosyl derivatives of piperazine. C. G. C. Catesby and A. M. Stephen (Univ. Cape Town). *J. Org. Chem.*, 1961, **26**, 601-603.

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PHYSICAL CHEMISTRY

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Distribution coefficient of the anionic thiocyanate complexes of metal cations. L. D. C. Bok and V. C. S. Schüler (Univ. Orange Free State, Bloemfontein). *J. S. African Chem. Inst.*, 1961, **14**, 45-49.

The system methanol-butanol-water-hydrocarbons. A. W. F. Middelberg, J. I. De Villiers, I. P. Marais, H. R. van der Walt and P. M. J. Réty (Production Dept., Sasol, Sasolburg). *S. African Ind. Chemist.*, 1961, **15**, **85-93.**

X-ray diffraction studies of thermally decomposed permanganates. P. J. Herley and E. G. Prout (Rhodes Univ., Grahamstown). *Nature*, 1960, **188**, 717-718.

The crystal structure of Mn_2Hg_5. J. F. de Wet (Nat. Chem. Res. Lab., Pretoria). *Acta Cryst.*, 1961, **14**, 733-738.

Packing of Spheres. J. R. Parrish (Nat. Chem. Res. Lab., Pretoria). *Nature*, 1961, **190**, 800.

Concentration polarization in electrodialysis -1. The electrometric measurements of interfacial concentration. B. A. Cooke (Nat. Chem. Res. Lab., Pretoria). *Electrochim. Acta*, 1961, **3**, 307-317.

Kirkendall effect in gases. L. Miller, P. C. Carman and E. A. Mason (Nat. Chem. Res. Lab., Pretoria). *Nature*, 1961, **191**, 375.

Heats of chemisorption of CO_2, CO and H_2 on zinc oxides with and without valency induction. P. M. G. Hart and F. Sebba (Univ. Cape Town). *Trans. Faraday Soc.*, 1960, **56**, 551-556.

Densitisation of explosives. P. W. Linder (African Explosives & Chem. Industries, Northrand). *Trans. Faraday Soc.*, 1961, **57**, 1024-1026.

BIOCHEMISTRY

Kaffircorn malting and brewing studies. Part VIII. Nutritive value of some kaffircorn products. M. C. Aucamp, J. J. Grieff, L. Novellie, B. Papendick, H. M. Schwartz and A. G. Steer. Part IX. Amino-acid composition of kaffircorn grain and malt. P. J. Horn and H. M. Schwartz (Nat. Chem. Res. Lab. Pretoria). *J. Sci. Fd. Agric.*, 1961, **12**, 449-456, 457-458.

The effect of large amounts of certain carbohydrates on the stability of free and combined lysine, histidine and orginine during acid hydrolysis. R. P. Bürke (Nat. Nutrition Res. Inst., Pretoria). *S. African J. Agric. Science*, 1961, **4**, 63-69.

Mitochondria of the Ehrlich ascites-tumour cell 2. Diphosphopyridine nucleotide—and triphosphopyridine nucleotide—dependent oxidation of isocitrate. A. O. Hawtrey and M. H. Silk (Liesbeek Cancer Res. Clinic, Cape Town) Biochem. J., 1961, **79**, 235-246.

Rutin from *Pachystigma pygmaeum*. A. L. Clingman (Nat. Chem. Res. Lab., Pretoria). *J. S. African Chem. Inst.*, 1961, **14**, 43-44.

Experiments on wool from copper-deficient sheep. Part III: The action of trypsin on oxidized wool. R. W. Burley and F. W. A. Horden (Nat. Chem. Res. Lab., Pretoria). *Textile Research J.*, 1961, **31**, 389-398.

Protein-bound azo dyes in the serum of rats fed 3′-methyl-4-dimethylaminoazobenzene. J. Dijkstra and F. J. Joubert (Nat. Chem. Res. Lab., Pretoria). *British J. Cancer*, 1961, **15**, 168-173.

The control of browning of white table wines. J. P. de Villiers (Nat. Chem. Res. Lab., Pretoria). *Amer. J. Enol.*, 1961, **12**, 25-30.

Chromatographic separation of biologically-active components from commercial trypan blue. J. Dijkstra (Nat. Chem. Res. Lab., Pretoria) and J. Gillman (Univ. Witwatersrand, Johannesburg). Nature, 1961, **191**, 803.

ANALYTICAL CHEMISTRY

The combination of ion exchange and spectrographic techniques for the estimation of trace elements in geological material. L. H. Ahrens, R. A. Edge and R. R. Brooks (Univ. Cape Town). *S. African Ind. Chemist*, 1961, **15**, 102-105.

An investigation of the reported interference by halogen acids in the determination of zinc by atomic absorption spectrophotometry. N. P. Finkelstein and A. V. Jansen (Central Metallurgical Lab., Johannesburg). *S. African Ind. Chemist*, 1961, **15**, 106-108.

An analytical procedure for the determination of platinum and palladium in certain ores. G. S. James (Central Metallurgical Lab., Johannesburg). *S. African Ind. Chemist*, 1961, **15**, 62-68.

Catalytic action of cuprous iodide. E. F. C. H. Rohwer (Univ. Stellenbosch). *S. African Ind. Chemist*, 1961, **15**, 22-26.

Some properties of a weakly acidic ion exchange resin. R. Arnold, Shirley MacIntosh and D. Reichenberg. (Univ. Cape Town). *S. African Ind. Chemist*, 1961, **15**, 30-32.

The absorption spectra of complexes of polyenic compounds with the Carr-Price reagent. C. F. Garbers, K. M. M. Kruse and T. Kleinsteuber (Nutrition Unit of C.S.I.R. Univ. Witwatersrand, Johannesburg and Nat. Phys. Res. Lab., Pretoria). *S. African Ind. Chemist*, 1961, **15**, 33-37.

The chemical concentration and spectrographic detrmination of certain minor trace metals in plant material. A Strasheim and D. J. Eve (Nat. Phys. Res. Lab., Pretoria). *J. S. African Chem. Inst.*, 1961, **14**, 1-15.

On the preparation of potassium bromide discs for infrared analysis. J. Morris and S. J. van der Walt (Iron and Steel Corp., Pretoria). *J. S. African Chem. Inst.*, 1961, **14**, 16-21.

A spray method for the simultaneous detection of iodide and iodinated amino-acids on filter paper chromatography. A. van Zyl and M. Bhaga (Univ. Cape Town). *Nature*, 1961, **191**, 275-276.

Use of carboxylic acid cation exchange resin in the analysis of copper in chromated wood preservative solutions. R. A. Edge (Univ. Cape Town). *Anal Chim. Acta.*, 1961, **24**, 143-145.

A separation of beryllium from aluminium, trivalent iron, yttrium, cerium, and the rare earths by cation exchange chromatography. F. W. E. Strelow (Nat. Chem. Res. Lab., Pretoria). *Anal Chem.*, 1961, **33**, 542-545.

Separation of trace elements in plant materials from ferric iron by cation exchange chromatography. F. W. E. Strelow (*Nat. Chem. Res. Lab., Pretoria*). *Anal Chem.*, 1961, **33, 994-996.**

INDUSTRIAL CHEMISTRY

The regeneration of cobalt-poisoned ion-exchange resins and their subsequent performance in a uranium plant. E. A. Nugent, P. Hoogervorst and R. E. Wheeler (West Rand Cons. Mines, Johannesburg) *J. S. African Inst. Mining and Met.*, 1960, **60**, 309-332.

The colorimetric determination of minute quantities of gold by means of o-tolidine. E. Frankel. *J. S. African Inst. Mining and Met.*, 1960, **60**, 367-370.

The colorimetric determination in merrill tail solutions at the Hartebeestfontein Gold Mines. C. R. Pugh and H. T. Tucker (Hartbeestfontein Gold Mining Co.). *J. S. African Inst. Mining and Met.*, 1960, **60**, 371-375.

A resin-in-pulp process using a floating resin technique for extracting uranium from acid leached pulp. E. H. D. Carman (Gold Fields of S. Africa, Johannesburg). *J. S. African Inst. Mining and Met.*, 1960, **60**, 647-662.

Recovery of lead from zinc plant residues by brine leaching and electrodeposition. D. D. Marsden (Central Metallurgical Lab., Johannesburg). *J. S. African Inst. Mining and Met.*, 1961, **61**, 522-534.

Analysis of ashes of South African coals. F. H. Kunstmann, L. B. Bodenstein and S. B. Gass (Fuel Res. Board, Pretoria). *J. S. African Inst., Mining and Met.*, 1960, **60**, 376-383.

Demineralization of sea water. J. R. Wilson (Nat. Chem. Res. Lab., Pretoria). *Research* 1961, **14**, 278-284.

SOME EQUILIBRIUM DISTRIBUTION COEFFICIENT VALUES FOR CATIONS IN NITRIC, SULPHURIC AND HYDROCHLORIC ACIDS USING AG SULPHONATED POLYSTYRENE RESINS

by

F. W. E. STRELOW

OPSOMMING

Die belangrikheid van die ewewigsverdelingskoeffisient, Kd, vir die beplanning van kromatografiese joonuitruilingkolomskeidings word beklemtoon. Tabelle van Kd-waardes in soutsuur, salpetersuur en swawelsuur met die hars AG 50W-X8 word aangetoon. Die invloed van die divinielbenseenkruisbinding van die hars op die Kd-waardes is ondersoek en 'n aantal resultate vir soutsuur- en salpetersuurmedia word weergegee.

SUMMARY

The importance of the equilibrium distribution coefficient, Kd, for the planning of chromatographic ion-exchange column separations is pointed out. Tables of Kd values in hydrochloric, nitric and sulphuric acid using the cation-exchange resin AG 50W-X8 are presented. The effect of the divinylbenzene crosslinking of the resin on the Kd values was examined and some results are shown for hydrochloric and nitric acid media.

INTRODUCTION

Ion-exchange and ion-exchange chromatography have gained extensive use in analytical and preparative chemistry during the past two decades. Already a wealth of separation procedures is available to the analytical chemist. Among the more spectacular achievements are: a sharp and simple separation of tantalum and niobium[1], quantitative separations of the rare earths[2] and preparative separations of pure hafnium from zirconium[3].

The most important and widely used ion-exchange materials are the artificial resins with a crosslinked polystyrene structure. Mayer and Tompkins[4, 5] applied the theoretical plate theory of the distillation process to the chromatographic ion-exchange column separation and developed the equation

$$\bar{v} = \text{Kd} \times [\text{Mass of dry resin in column in grams}] \quad \ldots\ldots\ldots\ldots \quad (1)$$

In this equation, $\bar{v}$ represents the volume of the eluting agent in millilitres which has to be passed through the column in order to elute the maximum of the elution peak, while Kd is the equilibrium distribution coefficient of the cation in question and is given by

$$\text{Kd} = \frac{\text{Equivalents of cation on resin} \times \text{Volume of aqueous phase in ml}}{\text{Equivalents of cation in aqueous phase} \times \text{Gram dry resin}} \quad [6, 7] \quad (2)$$

Equation (1) may be considered as the most important equation for the application of ion-exchange chromatography to analytical separations because it connects the equilibrium distribution coefficient, which can be determined very easily by a batch procedure, with the chromatographic column elution. Tompkins and Mayer[7] found good agreement between theoretical and experimental elution curves when the citrate complexes of the rare earths were eluted at traces concentrations from cation-exchange columns. Strelow[8] showed that equation (1) applies to the elution of cations

such as trivalent iron, divalent copper, calcium and aluminium with hydrochloric acid from cation-exchange columns, and it seems reasonable to assume that the equation is of general application.

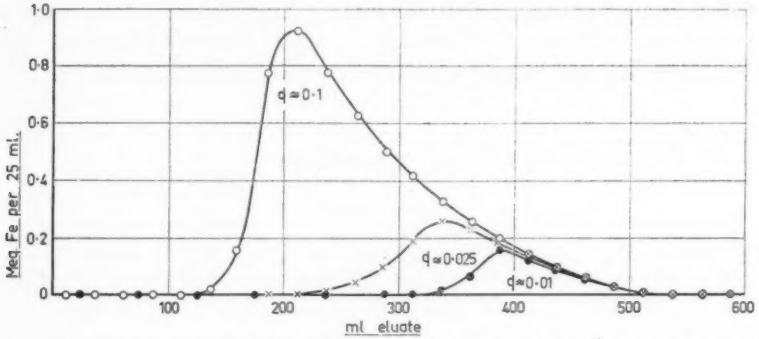

FIG. 1—Elution curves for different amounts of trivalent iron. Column of 12 g dry AG 50W-X8, 100 to 200 mesh, length 23 cm, diameter 1·25 cm, flow-rate 2·0-2·5 ml per minute, eluent 1N HCl.

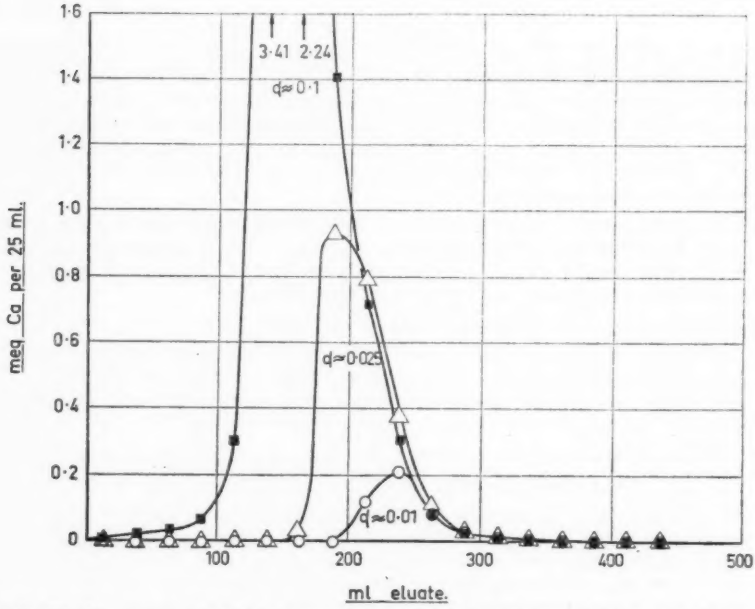

FIG. 2—Elution curves for different amounts of calcium. Column of 20 g dry AG 50W-X8, 100 to 200 mesh, length 45 cm, diameter 1·15 cm, flow-rate 1·2±0·2 ml per minute, eluent 2N HCl.

Equation (2) is valid only when the total amount of the cation present is less than about 3% of the total column capacity[5], but this does not detract from its value because the deviations in the experimental position of the peak maximum from that indicated by equation (2) are semi-quantitatively predictable and depend mainly on the Kd value of the cation, resin and eluent in question, and the ratio of amount of cation to column capacity. The influence of the latter ratio on the position and shape of the elution peak is demonstrated for ferric iron (Kd = 35·5 in N HCl) in Fig. 1, and calcium (Kd = 12·2 in 2N HCl) in Fig. 2. These Figs. show that the cations appear earlier in the eluate for higher column loads. The loading effect increases with increasing Kd values. Figs. 1 and 2 further demonstrate that the tails of the elution curves are stationary for the column loads investigated. This is very important because it implies that the appearance of the last traces of an element in the eluate is a constant for a column of given dimensions and a given eluent, and is determined mainly by the Kd value as long as the column is not seriously overloaded.

DISTRIBUTION COEFFICIENTS

From the above it becomes apparent that a table of numerical values of Kd for cations at different acid concentrations, or, alternatively, plots of Kd against concentration of eluent as supplied by Kraus *et al.*[9] for the chloride complexes in anion-exchange, should be of considerable value to the analytical chemist. While occasionally a limited number of distribution coefficients has been recorded in the literature,[10,11,12,13,14,15] no comprehensive investigation of equilibrium distribution coefficients of cations, with the aim of providing basic data for analytical separations, appears to have been attempted in the past, until one was undertaken in this Laboratory with the object of filling this gap at least partly.

As a result of this work, a table of Kd values in hydrochloric acid was prepared for 43 cations[8]. The numerical value of Kd in 1·0N hydrochloric acid for a ratio of total amount of cation to total resin capacity of q = 0·4 was chosen arbitrarily in order to arrange the cations in a selectivity sequence. Variations of Kd values with variations in the q ratio were determined, and relations between Kd values and elution curves and Kd values and tailing were investigated and discussed. The table of Kd values in hydrochloric acid is reproduced in Table I because it presents valuable basic information for the planning of cation-exchange separations and may be used together with the new tables for nitric and sulphuric acids.

In the present paper some results of the systematic investigation of Kd values in nitric and sulphuric acid media are reported. The same scheme was used to arrange the cations in selectivity sequences. Because a change in the crosslinking of the resin results in changes of Kd values which may become useful to the analytical chemist, Kd values for some cations in hydrochloric and nitric acids were dtermined at 2, 4, 8, 12 and 16 per cent crosslinking.

EXPERIMENTAL

The resin used was the AG 50 sulphonated polystyrene, processed from Dowex 50 by the BIO-RAD laboratories of Berkeley, California. The resin was used in the hydrogen form. It can be supplied in different degrees of crosslinkage, ranging from 1% to 16% divinylbenzene, and a large variety of particle sizes. It is very pure and normally has an inorganic ash content of 0·4 to 1·0 mg per 10 g dry resin.

Procedure to determine Kd values. An accurately weighed 2·5 gram portion of AG 50 resin of the desired crosslinkage, dried to constant weight at 105°C, was transferred to a 500 ml Erlenmeyer flask. Unless specially stated, the resin was of 100- to 200-mesh. Twenty ml of a standardized solution of the cation in 1N acid was added,

followed by 230 ml and/or the right amount of standardized acid to give a final volume of 250 ml of acid of the desired concentration. The flask was stoppered and shaken mechanically for 24 hours (48 hours for thorium) at room temperature. The resin was then filtered from the aqueous phase, care being taken to let it drain completely before the flask and filter was washed twice with distilled water. These washings were omitted in the case of the alkali metals. The amount of cation in the total aqueous phase plus washings was determined by an appropriate analytical method and, as a check, the amount in the resin was determined where possible after the resin had been ashed.

TABLE I

Kd values in hydrochloric acid. Resin AG 50W-X8

Cation	0·1N	0·2N	0·5N	1·0N	2·0N	3·0N	4·0N
Zr(IV)	$>10^5$	$>10^5$	$\sim10^5$	7,250	489	61	14·5
Th(IV)	$>10^5$	$>10^5$	$\sim10^5$	2,049	239	114	67
La(III)	$>10^5$	10^5	2,480	265·1	48	18·8	10·4
Ce(III)	$>10^5$	10^5	2,460	264·8	48	18·8	10·5
Y(III)	$>10^5$	$>10^4$	1,460	144·6	29·7	13·6	8·6
Ba(II)	$>10^4$	2,930	590	126·9	36	18·5	11·9
Al(III)	8,200	1,900	318	60·8	12·5	4·7	2·8
Sr(II)	4,700	1,070	217	60·2	17·8	10·0	7·5
Ga(III)	10^4	3,036	260	42·58	7·75	3·2	0·36
Ca(II)	3,200	790	151	42·29	12·2	7·3	5·0
Fe(III)	9,000	3,400	225	35·45	5·2	3·6	2·0
*Cr(III)	1,130	262	73	26·69	7·9	4·8	2·7
Ni(II)	1,600	450	70	21·85	7·2	4·7	3·1
Co(II)	1,650	460	72	21·29	6·7	4·2	3·0
Mg(II)	1,720	530	88	20·99	6·2	3·5	2·0
Mn(II)	2,230	610	84	20·17	6·0	3·9	2·5
Fe(II)	1,820	370	66	19·77	4·1	2·7	1·8
Cs(I)	182	99	44	19·41	10·4	—	—
U(VI)	5,460	860	102	19·20	7·3	4·9	3·3
Cu(II)	1,510	420	65	17·50	4·3	2·8	1·8
Zn(II)	1,850	510	64	16·03	3·7	2·4	1·6
Rb(I)	120	72	33	15·43	8·1	—	—
K(I)	106	64	29	13·87	7·4	—	—
Be(II)	255	117	42	13·33	5·2	3·3	2·4
Ti(IV)	$>10^4$	297	39	11·86	3·7	2·4	1·7
V(IV)	—	230	44	7·20	—	—	—
Na(I)	52	28·3	12	5·59	3·6	—	—
Li(I)	33	18·9	8·1	3·83	2·5	—	—
Sn(IV)	$\sim10^4$	45	6·2	1·60	1·2	—	—
Cd(II)	510	84	6·5	1·54	1·0	0·6	—
V(V)	13·9	7·0	5·0	1·10	0·7	0·2	0·3
Mo(VI)	10·9	4·5	0·3	0·81	0·2	0·4	0·3
Se(IV)	1·1	0·6	0·8	0·63	1·0	—	0·7
Bi(III)	Ppt.	Ppt.	<1·0	<1·0	<1·0	<1·0	<1·0
As(III)	1·4	1·6	2·2	3·81	2·2	—	—
Sb(III)	Ppt.	Ppt.	Ppt.	Ppt.	2·8	—	—
Pt(IV)	—	—	—	1·4	—	—	—
Au(III)	0·5	0·1	0·4	0·84	1·0	0·7	0·2
Hg(II)	1·6	0·9	0·5	0·28	0·3	0·2	0·2

*More than one cationic species present.

RESULTS AND DISCUSSION

Kd values in nitric acid. It was expected that with some cations such as trivalent ron, divalent mercury and cadmium, which form chloride complexes, there would

be marked differences between their Kd values in hydrochloric and in nitric acids. Smaller differences could be expected from other cations. Accordingly, a systematic study of Kd values in nitric acid was undertaken and some of the results are presented in Table II. The chloride complex-forming cations such as iron(III), mercury(II), and cadmium (II) have considerably higher Kd values in nitric acid than in hydrochloric acid[8]. This fact renders a separation of iron from divalent heavy metals such as copper, nickel, cobalt, zinc and manganese in nitric acid[16] feasible, and also allows the separation of cadmium and mercury from the same metals and many others in hydrochloric acid[17]. Barium, and to a lesser extent strontium and calcium, have lower Kd values in nitric acid because they show a stronger ion-association in this acid. This fact can be used for the quantitative separation of these alkaline earths from zirconium, a separation that is not successful in hydrochloric acid[18]. Aluminium, copper and the divalent heavy metals show slightly lower Kd values in hydrochloric acid. For zirconium, lanthanum and the rare earths, the Kd values are approximately the same in both acids, but zirconium has lower Kd values in hydrochloric acid at concentrations higher than N, probably because of the formation of a chloride complex.

TABLE II

Kd values in nitric acid. Resin AG 50W-X8

Cation	0·1N	0·2N	0·5N	1·0N	2·0N	3·0N	4·0N
Zr(IV)	$>10^4$	$>10^4$	$>10^4$	6,500	652	112	30·7
La(III)	$>10^4$	$>10^4$	1,870	267	47·3	17·1	9·1
Ce(III)	$>10^4$	$>10^4$	1,840	246	44·2	15·4	8·2
Y(III)	$>10^4$	$>10^4$	1,020	174	35·8	13·9	10·0
Hg(I)	$>10^4$	7,600	640	94	33·5	19·2	13·6
Al(III)	$>10^4$	3,900	392	79	16·5	8·0	5·4
Fe(III)	$>10^4$	4,100	362	74	14·3	6·2	2·7
Ba(II)	5,000	1,560	271	68	13·0	4·6	3·6
Sr(II)	3,100	775	146	44·0	8·8	6·1	4·7
Pb(II)	$>10^4$	1,420	183	35·7	7·5	5·8	4·5
Ca(II)	1,450	480	113	35·3	9·7	4·3	1·8
Cd(II)	1,500	392	91	32·8	10·8	6·8	3·4
Co(II)	1,260	392	91	28·8	10·1	6·1	4·7
Mn(II)	1,240	389	89	28·4	11·4	7·1	3·0
Ni(II)	1,140	384	91	28·1	10·3	8·6	7·3
Cu(II)	1,080	356	84	26·8	8·6	4·8	3·1
Zn(II)	1,020	352	83	25·2	6·5	4·6	3·6
Mg(II)	794	295	71	22·9	9·1	5·8	4·1
Tl(I)	173	91	41·0	22·3	9·9	5·8	3·3
Ag(I)	156	83	35·0	18·1	7·9	5·4	4·0
Hg(II)	4,700	1,090	121	16·9	5·9	3·9	2·8
Be(II)	553	183	52	14·8	8·0	4·5	2·8
Ti(IV)	1,410	461	71	14·6	8·5	4·5	3·4

Kd values in sulphuric acid. Some cations show marked complex-formation or ion-association in sulphuric acid, as is indicated by the activity coefficients of their sulphates[19]. This should have a very distinct influence on the numerical value of their Kd values in sulphuric acid in comparison with those in hydrochloric and nitric acids. Cations such as tetravalent thorium, zirconium and hexavalent uranium form relatively stable sulphate complexes, and should show this effect very distinctly. Kd values for some cations with AG 50W-X8 resin in sulphuric were therefore determined. The results in Table III show that the Kd values of tetravalent thorium and zirconium are very much lower in sulphuric acid than in hydrochloric acid of the same concentration. The effect is less pronounced but still very distinct in the

case of uranium. Cations of some divalent heavy metals on the other hand show in sulphuric acid definitely higher Kd values than in either hydrochloric or nitric acids. This may partially be due to the fact that the hydrogen ion concentration in sulphuric acid is lower than in the other two acids.

TABLE III

Kd values in sulphuric acid. Resin AG 50W-X8

Cation	0·1N	0·2N	0·5N	1·0N	2·0N	3·0N	4·0N
Al(III)	$>10^4$	8,300	535	126	27·9	10·6	4·7
Mn(II)	1,590	606	165	59	17·4	8·9	5·5
Fe(III)	$>10^4$	2,050	255	58	13·5	4·6	1·8
Th(IV)	$>10^4$	3,900	263	52	9·0	3·0	1·8
Ni(II)	1,390	588	140	46·0	16·5	6·1	2·8
Cu(II)	1,310	505	128	41·5	13·2	6·7	3·7
U(VI)	596	118	29·2	9·6	3·2	2·3	1·8
Ti(IV)	395	225	45·8	9·0	2·5	1·0	0·4
Zr(IV)	546	474	98	4·6	1·4	1·2	1·0

The influence of the degree of crosslinking on Kd values. The degree of crosslinking will influence the dimensions of the free spaces in the resin structure and thus the effective density of the sulphonic acid groups or the availability of the sulphonic acid groups at the most effective distance. Therefore changes in the Kd values have to be expected with change of the degree of crosslinking of the resin. The extent of change of Kd for a certain change in resin crosslinking may vary from one cation to another, especially when the cations are of different valency. Thus, analytical separations of cations may become feasible with one crosslinking while they are not possible with another. For this reason, Kd values were determined at constant hydrochloric acid concentration and constant ratio of cation to resin capacity for a number of cations, using AG 50 resins of different percentages of divinylbenzene crosslinking. The experiments were conducted at an amount of cation to resin capacity ration of q = 0·4, and the experimental procedures used were as described. The results are presented in Table IV. Kd values for some elements with AG 50 resins of different degrees of crosslinking have been determined in N nitric acid for comparative purposes. The results are presented in Table V.

TABLE IV

Variation of Kd in 1N HCl with variation in degree of crosslinking

Cation	2% DVB	4% DVB	8% DVB	12% DVB	16% DVB
Th(IV)	280	534	2,049	7,820	7,880
Zr(IV)	3,270	4,400	7,250	7,350	12,800
Ce(III)	62·3	99·0	265	362	371
Ba(II)	32·4	43·7	127	202	226
Sr(II)	19·9	26·8	60·2	72·2	78·6
Al(III)	29·5	39·4	60·8	71·1	58·2
Fe(III)	15·7	18·8	35·5	43·7	44·2
Ca(II)	17·4	22·7	42·3	54·6	58·2
Mg(II)	14·4	15·4	17·9	20·2	17·9
U(VI)	10·9	12·8	19·2	25·1	27·4
Cu(II)	11·2	12·7	17·5	20·4	19·8
Be(II)	7·9	10·2	13·3	20·9	18·6
K(I)	9·7	10·9	13·9	17·8	19·1

TABLE V

Variation of Kd in 1N HNO_3 with variation in degree of crosslinking

Cation	2% DVB	4% DVB	8% DVB	12% DVB	16% DVB
Cu(II)	10·8	14·1	26·8	30·9	29·6
Ca(II)	15·1	20·8	35·3	48·1	52·0
Sr(II)	17·0	21·9	44·0	59·1	65·5
Ba(II)	17·6	25·9	68·3	120·2	130·7

The results in Tables IV and V indicate a general trend of increase of Kd value with increasing divinylbenzene crosslinking.This increase is more pronounced for the higher Kd values. Zirconium is a noteworthy exception because its increase in Kd value with increase in crosslinking is considerably less than one would expect from its very high Kd value at low crosslinking. Some of the cations show a decrease of their Kd values when the crosslinking is increased from 12% to 16%. The most pronounced increase is produced by the increase of crosslinking from 4% to 8%. Since the effective crosslinking of a resin is probably not accurately reproducible, this would explain why different batches of resin of the same degree of crosslinking very often give slightly different sets of Kd values. Even a variation of the crosslinking of the order of a few tenths of a per cent will have a noticeable effect on the Kd values.

High crosslinking, as can be seen from the tables, has the advantage that favourable Kd values are obtained, because the Kd value scale of cations is stretched out. On the other hand it has the disadvantage that the exchange rates decrease and that sometimes the columns will have to be operated at lower flow speeds.

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REFERENCES

1 Kraus and Moore, *J. Am. Chem. Soc.*, 1949, **71**, 3855.
2 Tompkins, Kym and Cohn, *ibid.*, 1947, **69**, 2769.
3 Huffmann, and Lilly, *ibid.*, 1949, **71**, 4147.
4 Mayer and Tompkins, *ibid.*, 1947, **69**, 2866.
5 Cornish, *Analyst*, 1958, **83**, 634.
6 Schindewolf, *Angew. Chem.*, 1957, **69**, 226.
7 Tompkins and Mayer, *J. Am. Chem. Soc.*, 1947, **69**, 2859.
8 Strelow, *Anal. Chem.*, 1960, **32**, 1185.
9 Kraus and Nelson, International Conference on the peaceful uses of atomic energy, Geneva, 1955, paper No. 837.
10 Cabell, A.E.R.E. C/M 233.
11 Diamond, *J. Am. Chem. Soc.*, 1954, **76**, 1461.
12 Diamond, *ibid.*, 1955, **77**, 2978.
13 Khopkar and De, *Anal. chim. Acta*, 1960, **23**, 441.
14 Yamatera, *J. Inorg. Nucl. Chem.*, 1958, **7**, 299.
15 Yoshino and Kojima, *Japan Analyst*, 1955, **4**, 311.
16 Strelow, *Anal. Chem.*, in the press.
17 Strelow, *ibid.* 1960, **32**, 363.
18 Strelow, *ibid.*, 1959, **31**, 1974.
19 Robinson and Stokes, *Electrolyte Solutions*, Butterworths, London, 1955, pp. 491-501.

THE CONSTITUTION OF TWO NEW PHYSIOLOGICALLY-ACTIVE TRITERPENOIDS FROM *LIPPIA REHMANNI*

by

L. A. P. ANDERSON, W. T. de KOCK and P. R. ENSLIN

OPSOMMING

Twee nuwe triterpeensure is uit die wortelbas van *Lippia rehmanni* Pears geïsoleer en hulle struktuure bepaal as 22β-angeloieloksioleanolsuur en 22β-angeloieloksi-24-hidroksioleanolsuur.

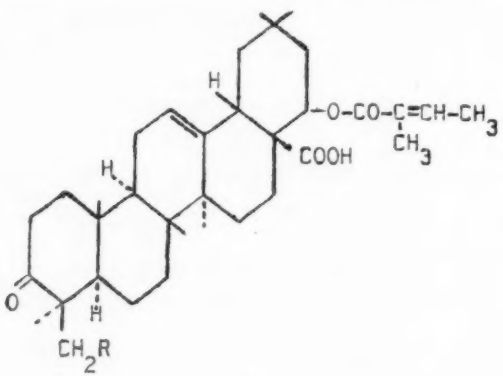
SUMMARY

Two new triterpenoid acids have been isolated from the root bark of *Lippia rehmanni* Pears and their structures determined as 22β-angeloyloxyoleanolic acid and 22β-angeloyloxy-24-hydroxy-oleanolic acid.

INTRODUCTION

The root bark of *Lippia rehmanni* Pears (Fam. Verbenaceae) contains a mixture of triterpenoid acids which is capable of producing a temporary bilirubinaemia accompanied by photosensitization when dosed orally to sheep. The syndrome produced closely resembles geeldikkop (tribulosis) in the lack of histological evidence of injury to the liver[1].

From the mixture of acids, Barton and de Mayo[2] (see also Rimington, Quin and Roets[3]) isolated icterogenin (I, R = OH) and rehmannic acid (lantadene A) (I, R = H) and determined their constitution and stereochemistry.



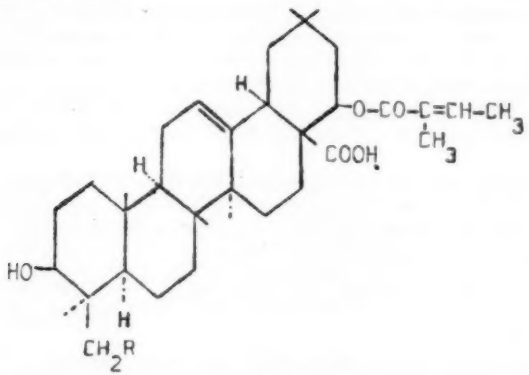
I

Rimington and co-workers[4] described a technique for administering these acids intraperitoneally to rabbits provided with external biliary fistulae and studied the changes in the quantity and composition of the bile. They found that the crude crystalline mixture of acids from the root bark of *Lippia rehmanni* was more active

than either pure icterogenin or rehmannic acid. This observation led us to examine the crude mixture for highly active minor constituents. The work was carried out in collaboration with Dr. J. M. Brown of the Division of Veterinary Services, Onderstepoort, who will report elsewhere on the physiological activity of the new substances isolated.

The crude crystalline acids were isolated in 1·3% yield by extraction with chloroform and crystallization from methanol. Paper chromatography showed that the mixture contained, in addition to icterogenin and rehmannic acid, at least two further minor components which could be isolated by repeated chromatography on acid-washed alumina and silica.

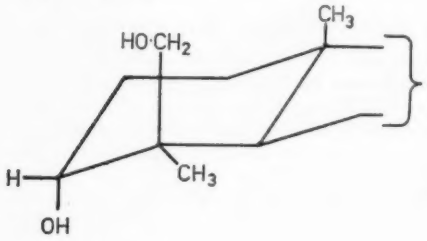
The one minor component, m.p. 296-298°, $[\alpha]_D + 70°$, is closely associated with rehmannic acid on paper chromatograms, and is a mono-basic acid of molecular formula $C_{35}H_{54}O_5$. It contains an $\alpha\beta$-unsaturated ester function (λ_{max} 209 mμ, ϵ 12,600) and gave a monoacetate, $C_{37}H_{56}O_6$, m.p. 254-256°, $[\alpha]_D + 63°$. Oxidation with 4N chromic acid in acetone[5] gave rehmannic acid in good yield. The configuration of the secondary hydroxy-group in position 3 was shown to be β(equatorial) on the basis of the following evidence. The substance showed a band at 1029 cm^{-1} characteristic of C(3)-equatorial hydroxy-groups in triterpenoids[6]. Furthermore, the change in molecular rotation on acetylation (Δ_1) is -12°. Reduction of rehmannic acid by the Meerwein-Ponndorf method gave two epimeric alcohols of which one was identical with the above new alcohol, and the other, m.p. 275-279°, $[\alpha]_D + 58°$, was characterized[6] as the 3α(axial)-alcohol by a band in its infrared spectrum at 1067 cm^{-1} and a $\Delta_1 = -160°$. Reduction of rehmannic acid with sodium borohydride gave only the 3β-alcohol in good yield. The new triterpenoid isolated from *Lippia rehmanni* is therefore 22β-angeloyloxyoleanolic acid (II, R = H). This substance has a very high physiological activity.



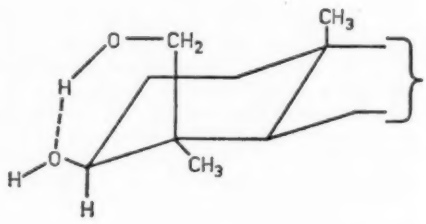
II

The second new substance isolated, $C_{35}H_{54}O_6$, m.p. 294-298°, $[\alpha]_D + 73°$ was identical with one of the two epimeric diols obtained on reduction of icterogenin with sodium borohydride. It gave a methyl ester, m.p. 202-205°, $[\alpha]_D + 72°$ and a diacetate, m.p. 240-242°, $[\alpha]_D + 63°$. The epimeric reduction product, $C_{35}H_{54}O_6$, m.p. 253-255°, $[\alpha]_D + 53°$, gave a methyl ester, m.p. 218-219°, $[\alpha]_D + 60°$ and a diacetate, m.p. 236-237°, $[\alpha]_D + 23°$. The configuration of the C(3)-hydroxy-groups in the two epimeric diols was established by an examination of the infrared spectra of the corresponding methyl esters. The spectrum of the methyl ester of m.p. 218-219°

showed only a single hydroxy-peak at 3641 cm^{-1}. The conformation of ring A of this epimer is therefore that shown in partial structure (III) in which the axial C(3)-hydroxy-group is too far removed from the C(24)-hydroxy-group of the axial C(4)-hydroxymethyl-group for intramolecular hydrogen-bonding. The methyl ester of m.p. 202-205° gave, in addition to a sharp peak at 3627 cm^{-1}, a broad peak at 3552 cm^{-1} indicating a bonded hydroxy-group[7], and has therefore partial structure (IV) with an equatorial 3-hydroxy-group. The second new triterpenoid acid from *Lippia rehmanni* has therefore the structure and stereochemistry as shown in II (R = OH).



III



IV

EXPERIMENTAL

Unless specified to the contrary, $[\alpha]_D$ refers to chloroform, ultraviolet absorption to ethanol, and infrared spectra to chloroform solutions. All infrared spectra were determined on a Perkin-Elmer Model 21 spectrometer, except the measurements of bonded and non-bonded hydroxy-groups which were determined in carbon tetrachloride solution on a Perkin-Elmer Model 221 spectrometer. Acid-washed alumina was perpared by washing Peter Spence alumina consecutively with dilute hydrochloric acid, hot water, and ethanol and reactivating it at 150° for 24 hr. The silica used for chromatography was obtained from Frederick Smith Chemical Co., 50-200 mesh.

Paper chromatography. Solvent system A:* Whatman No. 3MM paper was impregnated with a 30% solution in acetone of the upper phase of the solvent mixture: octanol-*iso*amyl alcohol-formamide-10% aqueous diethylamine (3:1:1:4) and the paper developed for 16 hr with the lower phase.

Solvent system B:† Whatman No. 3MM paper was impregnated with a 50% solution of formamide in ethanol containing 10% diethylamine and the paper developed with benzene for 1½ hr.

The chromatograms were dried at 120° and drawn through a 20% solution of antimony trichloride in dry chloroform containing 1% antimony pentachloride. Coloured spots developed on heating at 100° for 1 min.‡ The average Rf-values found in solvent systems A and B respectively are: 22β-angeloyloxyoleanolic acid (0·24, 0·64) 22β-angeloyloxy*epi*oleanolic acid (0·24, 0·70), rehmannic acid (0·33, 0·86), 22β-angeloyloxy-24-hydroxyoleanolic acid (0·56, 0·21), icterogenin (0·88, 0·22), 22β-angeloyloxy-24-hydroxy*epi*oleanolic acid (0·88, 0·03).

*A modification of that described by Tschesche and Poppel[8].

†The help of Mr. D. F. Gouws is gratefully acknowledged.

‡The helpful suggestions of Dr. G. Snatzke, Chemische Staatsinstitut, Hamburg, are gratefully acknowledged.

Extraction and separation of crude acids. Dried, ground root bark (9 kg) was extracted three times consecutively with boiling chloroform. Evaporation of the extract gave a foam (400 g) which was crystallized from methanol to yield a crude crystalline product (126 g). Paper chromatography (solvent system A) showed spots corresponding to icterogenin (Rf 0·88) and rehmannic acid (Rf 0·33) and in addition two further spots at Rf 0·24 and 0·56.

The crude mixture (21 g) was separated by chromatography on acid-washed alumina (2 kg) and the composition of individual fractions determined by paper chromatography (solvent system A). Elution of the column with 99:1 benzene-ethanol (3 l) gave an oil (109 mg) after which further elution with the same solvent (4 l) gave fractions (4.03 g) containing rehmannic acid (Rf 0·33) and a substance of Rf 0·24 (combined fraction A). Elution with benzene-ethanol (97:2, 5l; 95:5, 4 l.) gave fractions (13·01 g) containing mainly icterogenin (Rf 0·88). Crystallization from chloroform-methanol and chloroform-ether gave pure icterogenin (9·6 g), m.p. 240-242°. Further elution of the column with 85:15 benzene-ethanol (3·5 l.) gave fractions (1·51 g) enriched in a substance of Rf 0·56 (combined fraction B). Later fractions obtained with the same solvent consisted of a complex mixture of substances.

22β-angeloyloxyoleanolic acid. Combined fraction A (6·3 g) was rechromatographed on silica (1·2 kg). Elution of the column with 95:5 benzene-ether (13 l) gave fractions (4·34 g) from which rehmannic acid (3·8 g), m.p. 284-286°, was isolated by crystallization from chloroform-methanol. Elution with 9:1 benzene-ether (1·5 l) gave mixed fractions which were discarded after which 7:3 benzene-ether (3 l) eluted fractions (948 mg) strongly enriched in the substance of Rf 0·24 (solvent system A). Rechromatography on silica (250 g) and crystallization from chloroform-methanol and chloroform-ether gave 22β-angeloyloxyoleanolic acid (314 mg), m.p. 296-298°, $[\alpha]_D$ + 70° (*c* 1·0), λ_{max} 209 mμ (ε 12,600), ν_{max} 1029 cm^{-1} (equatorial 3-hydroxy-group), negative Zimmermann colour test [2] (Found: C, 75·5; H, 9·8. Calc. for $C_{35}H_{54}O_5$: C, 75·8; H, 9·8%).

Acetylation in boiling acetic anhydride-pyridine gave the acetate, m.p. 254-256° (from chloroform-methanol), $[\alpha]_D$ + 63° (*c* 1·04) (Found: C, 74·0; H, 9·4. Calc. for $C_{37}H_{56}O_6$: C, 74·5; H, 9·5%).

Oxidation to rehmannic acid. The above alcohol of m.p. 296-298° (20 mg) was dissolved in acetone (8 ml, stabilized over potassium permanganate) and a solution (0·08 ml) of chromium trioxide (0·67g) in 11·5% sulphuric acid (5 ml) added slowly at 5-10° over a period of 15 min. After addition of methanol (1 ml) and water (10 ml), the product was isolated with chloroform and crystallized from chloroform-methanol to give rehmannic acid (12 mg), identified by m.p., mixed m.p., paper chromatography and its infrared spectrum.

Reduction of rehmannic acid by the Meerwein-Ponndorf method. A solution of rehmannic acid (990 mg) and aluminium *iso*propoxide (1·5 g) in *iso*propanol (22 ml) was distilled slowly through a column until the distillate was free from acetone (*ca* 4 hr). After removal of the solvent *in vacuo*, the residue was washed with dilute hydrochloric acid and the product isolated with 1:1 chloroform-ether. The crude product showed two spots (Rf 0·70, mauve and 0·64, blue) on paper chromatograms (solvent system B). The mixture was separated by chromatography on silica (200 g). Elution with 95:5 benzene-ether (1·5 l) gave fractions (409 mg) containing only the substance of Rf 0·70. Further elution of the column with benzene-ether (95:5, 1·5 l; 9:1, 1·5 l) gave fractions (537 mg) enriched in the substance of Rf 0·64. This fraction was purified by rechromatography on silica (110 g) to yield a further 120 mg of the

substance of Rf 0·70 and 22β-angeloyloxyoleanolic acid (130 mg from chloroform-methanol), Rf 0·64. The latter was identical (m.p., mixed m.p., infrared spectrum) with the new substance isolated from *Lippia rehmanni*.

Crystallization of the fractions containing the substance of Rf 0·70 gave 22β-angeloyloxy*epi*oleanolic acid, m.p. 275-279° (from chloroform-methanol), $[\alpha]_D$ + 58° (*c* 0·8), ν_{max} 1067 cm^{-1} (axial 3-hydroxy-group) (Found: C, 75·5; H. 9·7. Calc. for $C_{35}H_{54}O_5$: C, 75·8; H, 9·8%).

Acetylation in boiling acetic anhydride-pyridine gave the acetate, m.p. 287-288° $[\alpha]_D$ + 27° (*c* 0·83) (Found: C, 74·4; H, 9·6. Calc. for $C_{37}H_{56}O_6$: C, 74·5; H, 9·5%).

Reduction of rehmannic acid with sodium borohydride. Rehmannic acid (1 g) in methanol (500 ml) was treated with sodium borohydride (500 mg) in methanol (20 ml) for 30 min. The reaction mixture was poured into water (500 ml), acidified with dilute hydrochloric acid and the product isolated with ether. Crystallization from chloroform-methanol and chloroform-ether gave 22β-angeloyloxyoleanolic acid (600 mg), m.p. 294-298°, $[\alpha]_D$ + 67° (*c* 1·0) (Found: C, 75·8; H, 9·6. Calc. for $C_{35}H_{54}O_5$: C, 75·8; H, 9·8%).

Acetylation in boiling acetic anhydride-pyridine gave the acetate, m.p. 255-257°, $[\alpha]_D$ + 64° (*c* 1·0) (Found: C, 74·05; H, 9·4. Calc. for $C_{37}H_{56}O_6$: C, 74·5; H, 9·5%). This substance was identical (m.p., mixed m.p. and infrared spectrum) with the acetate of naturally occurring 22β-angeloyloxyoleanolic acid.

22β-*angeloyloxy*-24-*hydroxyoleanolic acid.* Combined fraction B (from chromatogram of crude acids) was rechromatographed on acid-washed alumina (150 g). Elution with benzene-ethanol (98:2, 1 l; 95:5, 500 ml) gave icterogenin (590 mg). Further elution of the column with benzene-ethanol (95:5, 1·9 l) gave fractions (800 mg) containing the substance of Rf 0·56 still contaminated with icterogenin. Rechromatography on acid-washed alumina (100 g) finally gave a few fractions (310 mg) which showed only the spot at Rf 0·56 on paper chromatograms (solvent system A). Crystallization from chloroform-methanol gave fine needles (140 mg), m.p. 294-298°, $[\alpha]_D$ + 73° (*c* 1·5 in ethanol) (Found: C, 73·5; H, 9·7. Calc. for $C_{35}H_{54}O_6$: C, 73·6; H, 9·5%).

Acetylation in boiling acetic anhydride-pyridine gave the acetate, m.p. 240-242°, $[\alpha]_D$ + 63° (*c* 0·8).

Reduction of icterogenin with sodium borohydride. Icterogenin (2 g) in methanol (500 ml) was treated with sodium borohydride (680 mg) in methanol (30 ml) and kept at room temperature for 5 hr. After dilution with water (500 ml) and acidification with dilute hydrochloric acid, the product was isolated with ether. Paper chromatography (solvent system A) showed it to consist of two components of Rf 0·56 and 0·88. The mixture was separated by chromatography on acid-washed alumina (300 g). Elution with 1:1 chloroform-benzene containing 3% ethanol (1·3 l) gave fractions (570 mg) containing mainly the component of Rf 0·56. Crystallization from ethanol gave fine needles (400 mg), m.p. 294-297°, $[\alpha]_D$ + 74° (*c* 0·5 in ethanol), λ_{max} 208 mμ (ϵ 12,500) (Found: C, 73·5; H, 9·4. Calc, for $C_{35}H_{54}O_6$: C, 73·6; H, 9·5%). The identity of this substance and naturally occurring 22β-angeloyloxy-24-hydroxyoleanolic acid was established by mixed m.p., paper chromatography and their infrared spectra. Treatment with diazomethane in methanol-ether gave the methyl ester as fine needles (from methanol), m.p. 202-205°, $[\alpha]_D$ + 72° (*c* 1·2), ν_{max}. (*c* 0·2 in carbon tetrachloride) 3627 and 3552 (broad) cm^{-1} (free and bonded hydroxy-groups respectively) (Found: C, 73·6; H, 9·3. Calc. for $C_{36}H_{56}O_6$: C, 73·9; H, 9·7%). Acetylation with boiling acetic anhydride-pyridine gave the diacetate (from methanol), m.p. 240-242°, $[\alpha]_D$ + 63° (*c* 1·6) (Found: C, 71·4; H, 8·9. Calc, for $C_{39}H_{58}O_8$: C, 71·5; H, 8·9%).

This acetate gave an infrared spectrum identical with that of the acetate of naturally occurring 22β-angeloyloxy-24-hydroxyoleanolic acid.

Further elution of the column with the same solvent (500 ml) gave mixed fractions. The second component of Rf 0·88 was then eluted with 1:1 chloroform-benzene containing 8% ethanol (1 l) and crystallized from ethanol and then from ether to give needles (500 mg), m.p. 253-255°, $[\alpha]_D + 53°$ (*c* 0·5 in ethanol), λ_{max} 208 mμ (ϵ 12,100) (Found: C, 73·5; H, 9·5. Calc. for $C_{35}H_{54}O_6$: C, 73·6; H, 9·5%). Treatment with diazomethane gave the methyl ester (from chloroform-ether), m.p. 218-219°, $[\alpha]_D + 60°$ (c 1·3), ν_{max} (*c* 0·2 in carbon tetrachloride 3641 cm^{-1} (non-bonded hydroxy-groups) (Found: C, 73·8; H, 9·7. Calc. for $C_{36}H_{56}O_6$: C, 73·9; H, 9·7%). Acetylation in boiling acetic anhydride-pyridine gave the diacetate (from methanol), m.p. 236-237°, $[\alpha]_D+$ 23° (c 0·5 in ethanol) (Found: C, 71·6; H, 8·8. Calc. for $C_{39}H_{58}O_8$: C, 71·5; H, 8·9%).

The authors wish to thank Mr. K. I. Jones for the micro-analyses. This paper is published by permission of the South African Council for Scientific and Industrial Research, and the Director of Veterinary Services, Onderstepoort.

REFERENCES

[1] Brown, *J. S. African Vet. Med. Assoc.*, 1959, **30**, 97.
[2] Barton and de Mayo, *J. Chem. Soc.*, **1954**, 887, 900.
[3] Rimington, Quin and Roets, *Onderstepoort J. Vet. Sci. Animal Ind.*, 1937, **9**, 225.
[4] Heikel, Knight, Rimington, Ritchie and Williams, *Proc. Royal Soc.* (London), B, 1960, **153**, 47.
[5] Bowden, Heilbron, Jones and Weedon, *J. Chem. Soc.*, **1946**, 39; see also Djerassi, Engle and Bowers, *J. Org. Chem.*, 1956, **21**, 1547.
[6] Allsop, Cole, White and Willix, *J. Chem. Soc.*, **1956**, 4868.
[7] Cole and Müller, *J. Chem. Soc.*, **1959**, 1224.
[8] Tschesche and Poppel, *Chem. Ber.*, 1959, **92**, 320.

THE CARBON-NITROGEN RATIO OF SOME SOILS IN THE MAIZE-GROWING AREAS OF SOUTH AFRICA

by

G. STIVEN

OPSOMMING

Dit is gevind dat die totale koolstof goed korreleer met die totale stikstof in die grondmonsters wat geneem is van die mielielande in Transvaal en die Oranje-Vrystaat. Daar is ook gevind dat die gemiddelde C:N-verhouding van die grond beduidend groter is as 12 ('n waarde wat algemeen aangeneem word as die ,,ewewig" waarde vir die C:N-verhouding van grond). Verder is dit duidelik van die regressie van koolstof teenoor stikstof dat die C:N-verhouding geensins konstant is nie, maar vermeerder met die vermeerdering van koolstof en stikstof, en benader waardes van 17:1 vir die boonste grondlaag en 16·5:1 vir die grond direk onder die ploeglaag.

Die manier waarop die koolstofgehalte verander met vermeerdering van die stikstofgehalte dui aan dat die byvoeging van stikstofbevattende kunsmis sal, oor die algemeen, deur die grondmikroflora en die groeiende gesaaides, opgeneem word.

SUMMARY

Data for the total carbon have been found to be well correlated with those of total nitrogen in soil samples taken from maize lands in the Transvaal and Orange Free State. The average C:N ratio of these soils has been found to be significantly greater than 12, a value generally accepted as the "equilibrium" value for the C:N ratio of soils. Furthermore, it is clear from the regression of carbon on nitrogen that the C:N ratio is not in fact constant, but increases with increasing carbon and nitrogen, approaching values of 17:1 for the topsoil and 16·5:1 for soil immediately below the plough layer.

The manner in which the carbon levels change with increasing levels of nitrogen indicates that applications of fertilizer nitrogen will, in general, be shared by the soil microflora and the growing crop.

INTRODUCTION

Data of the carbon:nitrogen ratios of South African soils have been reported by van der Merwe.[1] These have included values for soils found in the maize-growing areas of the Transvaal and Orange Free State. Table 1 shows the districts in which the various profiles were located, the depths of the horizons, and the C:N ratios. The profiles were all situated on virgin lands.

The average value of the C:N ratio of the upper horizons is 12·46:1, and for the lower horizons 11·35:1.

The C:N ratios of soils in other parts of the world have been reported by many workers. McClean[2] showed that the C:N ratio of many British soils lay between 8·5 and 11·4. Robinson[3] has stated that in temperate regions the ratio approaches 10:1, and Ensminger and Pearson[4] seem to think that an average value of 12:1 would apply to most soils.

It has long been known that the C:N ratio is relatively constant and independent of treatment.[2, 4, 5, 6] If organic material with a C:N ratio widely different from that of a soil is added to that soil, the microbial life in the soil will decompose the material until a residue remains having a C:N ratio equal to that of the soil. The C:N ratio is thus altered only temporarily by cropping and cultivation practice.[5, 6, 7]

The C:N ratio has been used as a means of characterising the organic matter of a soil.[5, 6] Soils in different areas often have different C:N ratios but the reasons for these differences are not clearly understood. In general, the differences which do occur seem to be correlated with climatic conditions, especially temperature and rainfall distribution. It is also known to change with depth.[3, 5, 6]

TABLE I

C:N ratios of soils reported by van der Merwe[1]

District	Depth of horizon	C:N ratio	Depth of horizon	C:N ratio
Senekal, O.F.S.	0″ — 8″	11·6:1	8″ — 14″	10·2:1
Winburg, O.F.S.	0″ — 10″	10·7:1	10″ — 25″	7·9:1
Bethlehem, O.F.S.	0″ — 10″	12·6:1	10″ — 19″	11·2:1
Hoopstad, O.F.S.	0″ — 16″	11·3:1	16″ — 26″	14·0:1
Schweizer Reneke, Tvl. ..	0″ — 7″	8·8:1	7″ — 24″	8·0:1
Bethal, Tvl.	0″ — 8″	15·1:1	8″ — 18″	13·2:1
Ermelo, Tvl.	0″ — 10″	15·6:1	10″ — 22″	15·5:1
Delmas, Tvl.	0″ — 11″	14·1:1	11″ — 30″	11·0:1

Many of the soils in the maize-growing areas of South Africa have been cropped continuously to maize for a number of years, often as many as 50, and widely differing methods of cultivation and fertilizing have been used. Phosphatic fertilizers have been used almost exclusively on the maize crops, so that the soils in these areas have had to supply the crops' needs for nitrogen entirely. The C:N ratio of these soils could conceivably be altered, temporarily at least, by such practices.

EXPERIMENTAL

Soil samples from experimental sites in maize lands in the Transvaal and Orange Free State were collected in September, 1958, just prior to the planting of the crop. The number of sites sampled in each province is shown in Table II. Two composite samples were taken from each site; one from the 0-6″ horizon, and one from the 6″—12″ horizon (called respectively for brevity the top- and subsoils) and analysed for total carbon by a procedure similar to Walkley and Black's rapid titration method, and for total nitrogen by the Kjeldahl digestion procedure.

CORRELATION AND REGRESSION ANALYSES

Correlation and regression of values of total carbon and total nitrogen in the topsoil. These values were found to be well correlated. The coefficient of correlation ($r = 0.9376$) was found to be highly significant ($P > 99{\cdot}9\%$). Fig. 1 shows the experimental points plotted on a scatter diagram.

Correlation and regression of values of total carbon and total nitrogen in the subsoil. These values were found to be well correlated and the coefficient of correlation ($r = 0{\cdot}9233$) was found to be highly significant. Fig. 2 shows these values plotted on a scatter diagram. The standard errors calculated for the slopes and the intercepts for the regression equations in Figs. 1 and 2, show that the intercepts

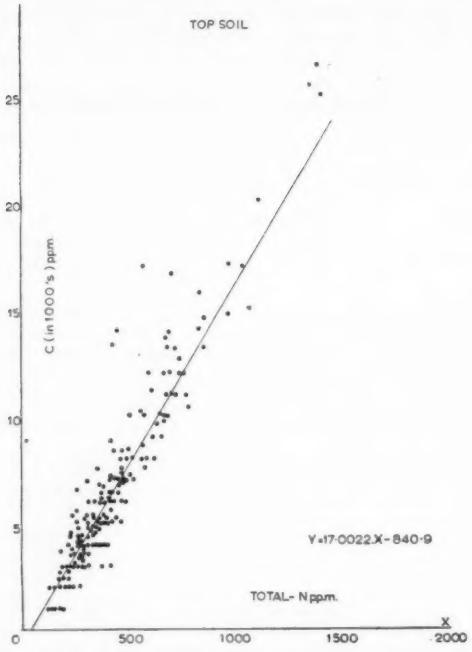


FIG. 1—Relationship between the carbon and nitrogen contents in the topsoil

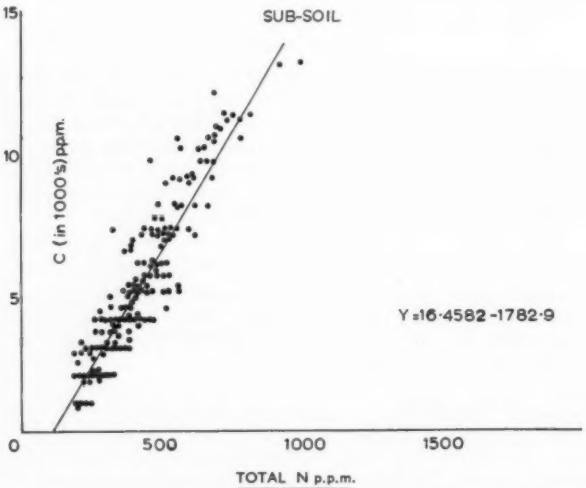


FIG. 2—Relationship between the carbon and nitrogen contents in the subsoil

are highly significantly different from 0(P>99·9%) and the slopes are significantly different from 14 and 12 (P>99·9%).

TABLE II

Distribution of the experimental sites

Area No.	Locality	No. of experimental sites in each locality
1	Western Transvaal	74
2	N.W. Free State	71
3	Eastern Tvl. Highveld	62
4	Eastern Orange Free State	31
Total		238

It may be concluded from the above analyses that changes in the total nitrogen status will result in relative changes in the total carbon status both in the top- and subsoils.

Table III summarises the results of the correlation and regression analyses.

TABLE III

Some parameters of the regression equations

Fig. No.	Regression equation	Standard errors		t values	P
1 Topsoil No. of obs. 238	Y=17·0022X−840·9 Y=total carbon (p.p.m.) X=total nitrogen (p.p.m.)	Intercept	207·05	3·8	>99·9%
		Slope	0·4105	For slope different from 12, 12·18	>99·9%
				For slope different from 14, 7·31	>99·9%
2 Subsoil No. of Obs. 238	Y=16·4582X-1782·9 Y=total carbon (p.p.m.) X=total nitrogen (p.p.m.)	Intercept	348·24	4.96	>99·9%
		Slope	0·2752	For slope different from 12, 18·17	>99·9%
				For slope different from 14, 10·91	>99·9%

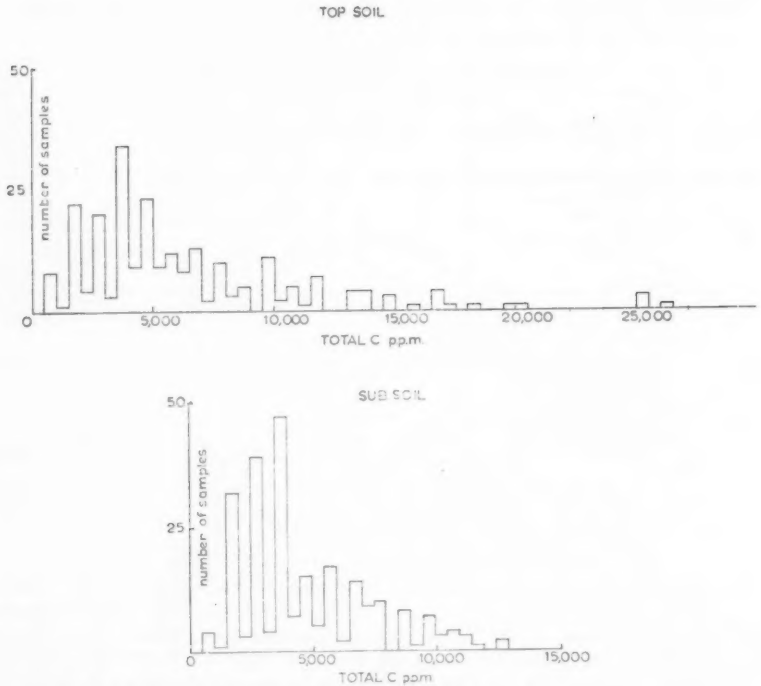
APPARENT CHANGES IN THE C:N RATIOS

The apparent change of the average value of the C:N ratio in the topsoil. The mean value of the C:N ratio in the topsoil was found to be 14·26:1 with a standard error of 0·2481. This mean was found to be highly significantly different from the mean value of 12.46:1 calculated from van der Merwe's data[1] so that from these observations it may be inferred that there has been an apparent change in the average C:N ratio in the topsoils of the maize-growing areas of the Transvaal and Orange Free State.

The average value of the C:N ratio in the subsoil. The mean value of the C:N ratio in the subsoil was found to be 11·56 with a standard error of 0·1926. This mean value is not significantly different from that calculated from van der Merwe's data,[1] although the two figures are not strictly comparable because the horizons sampled by him were deeper than those mentioned in this paper. This implies that the practices used by farmers have brought about no apparent change in the average value of the C:N ratio of the subsoils in those areas.

Dependence of C:N ratio on the levels of carbon and nitrogen in cultivated soils. From the regression of carbon on nitrogen it can be seen that any change in the nitrogen level will result in a 17 fold change in the carbon level in the topsoil, and a 16·5 fold change in the subsoil.

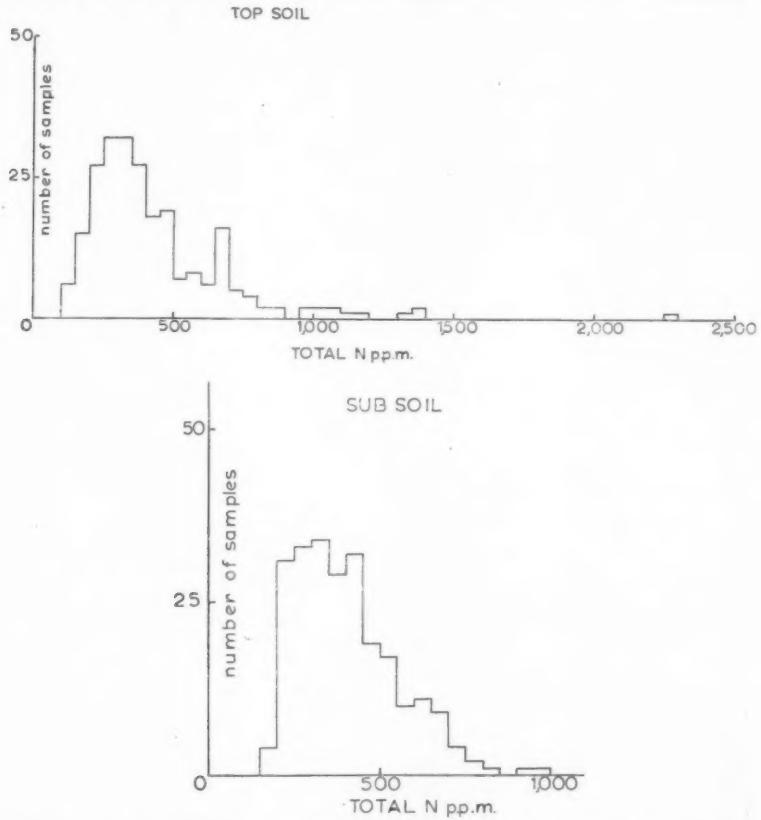
With increasing levels of nitrogen and carbon the C:N ratio approaches a value of 17:1 in the topsoil, and a value of 16·5:1 in the subsoil; with decreasing levels of nitrogen and carbon, the ratio approaches O, for the regression analysis reveals that a hypothetical situation could obtain where a soil could contain no carbon but still have small quantities of nitrogen. It is therefore inferred that the C:N ratio is not constant but is dependent on the levels of carbon and nitrogen in a soil.



FIGS. 3 and 4—Histogtams showing the distribution of the carbon contents in the top and subsoils

Assignable causes for the fluctuating levels of carbon and nitrogen in cultivated soils. Changes in the levels of carbon and nitrogen were shown experimentally by Pinck, Allison and Gaddy[7] when they grew five successive crops on soils treated with increasing amounts of nitrogen. Those not treated showed a carbon loss of 7·85% whereas those treated with a total of 800 lb. N per acre showed a 19·71% gain. Furthermore, from 46 to 75% of the added nitrogen was not recovered by the five successive crops but most of it was held in the soil.

It has been clearly shown by the authors cited in references[8, 9, 10, 11] that by increasing the carbon content of a soil, nitrogen increasingly is immobilised, and consequently it becomes less available to a growing crop. A possible explanation of this phenomenon arises from the work of Quastel and Scholefield.[12] When nitrogenous fertilizers are added to a soil they usually undergo rapid nitrification. Nitrification is the result of the proliferation of bacterial cells of the *Nitrosomonas* and *Nitrobacter* groups, and is usually accompanied by the evolution of large quantities of carbon dioxide. The evolution of carbon dioxide leads to the proliferation of other



FIGS. 5 and 6—Histograms showing the distribution of the nitrogen contents in the top- and subsoils

microbial groups, probably mostly autotrophs and possibly some heterotrophs, which assimilate the carbon from the carbon dioxide and much of the mineral nitrogen produced by the nitrifying bacteria. This leads to a change in the levels of carbon and nitrogen, with the change in carbon being much more rapid than that of nitrogen because plant bodies usually contain approximately about 30 times more carbon than nitrogen. If no further additions of nitrogen are made, the activities of the nitrifying bacteria are considerably reduced. The reduction in output of mineral nitrogen and carbon dioxide must in turn lead to the death and decay of most of the autotrophs with a consequent release of nitrogen, which can be taken up by the growing crop, and carbon which probably goes off into the atmosphere as carbon dioxide.

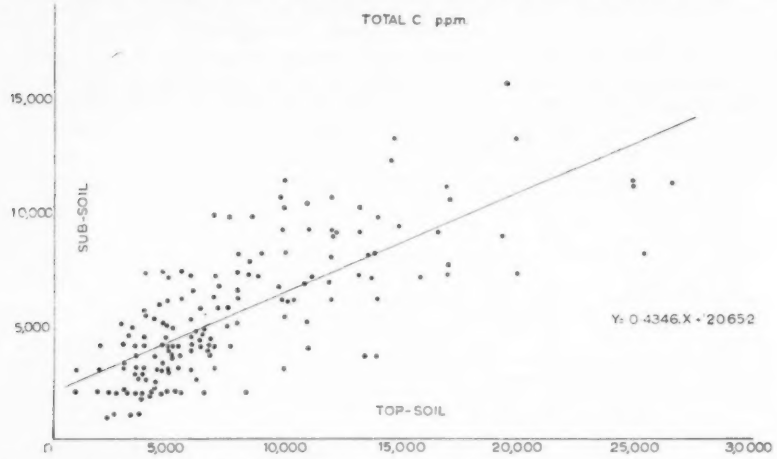


FIG. 7—Relationship between the carbon content in the topsoil and that in the subsoil

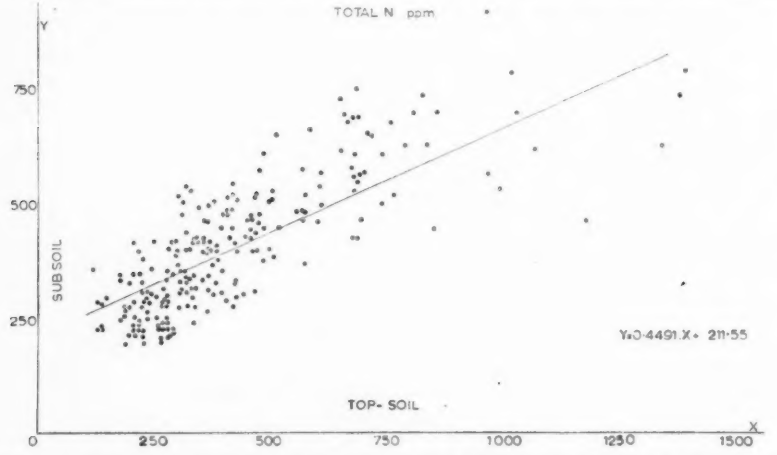


FIG. 8—Relationship between the nitrogen content in the topsoil and that in the subsoil

The changes in the levels of carbon and nitrogen under these conditions are probably very rapid when compared to the situation where no nitrogenous fertilizers are added to a cultivated soil. Here the nitrifying bacteria have to rely for their sources of energy on the ability of other groups of microflora to decompose the organic matter complex.

DISTRIBUTION OF THE VALUES OF TOTAL CARBON AND TOTAL NITROGEN

Figs. 3, 4, 5 and 6 demonstrate how the values of these two constituents were distributed among the soil samples, and offer an idea of the amounts found. Most of the soils analysed had total nitrogen contents lying between 250 p.p.m. and 500 p.p.m. in both the top- and subsoils. The corresponding values for total carbon were about 2,500 to 7,500 p.p.m. for the topsoils, and 1,500 to 5,000 p.p.m. for the subsoils.

Total carbon or nitrogen measured in the topsoil was found to be well correlated with that measured in the subsoil for (Figs. 7 and 8), so that in general if changes in value are measured in these two constituents in the topsoil relative changes will probably have occurred in the subsoil. The correlation coefficients were respectively *O*·7718 (Fig. 7) and *O*·7840 (Fig. 8), which were both statistically significant at the $P > 99{\cdot}9\%$ level of probability.

The regression coefficients shown in Figs. 7 and 8 indicate that both for carbon and nitrogen large changes in their status in the topsoil will only be reflected by small changes in the subsoil.

CONCLUSION

It is concluded that the average C:N ratio in the plough-layer of the soils of the maize-growing areas of the Transvaal and Orange Free State is 14:1 and moreover that there is a real difference between this value and the commonly accepted one of 12:1. This probably indicates a change in the character of the organic matter of the soil and may have been brought about by the cropping and cultivation methods used by farmers, and is probably of a temporary nature. Nevertheless, the consequences of this change should not be overlooked. It implies that nitrogen, both native to a soil, and applied, can be immobilised[7, 8, 9, 10, 11] and temporarily rendered only slowly available to the growing crop.

It is inferred that the C:N ratio is not constant but is dependent on the levels of carbon and nitrogen, and the value of this ratio varies between the limits of 0 and 7:1 in the topsoil, and 0 and 16·5:1 in the subsoil.

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REFERENCES

[1] van der Merwe, *Soil groups and sub-groups of South Africa*, Government Printer, Pretoria, 1941.
[2] McClean, *J. Agric. Sci.*, 1930, **20**, 348.
[3] Robinson, *Soils, their origin, constitution, and classification*, Thomas Murby & Co., London, 1949, p. 219.
[4] Ensminger and Pearson, *Advances in Agronomy* Vol. II, Academic Press, New York, 1950, p. 89.
[5] Russell, *Soil conditions and plant growth*, Longmans, Greens & Co., London, 1950, p. 27 .
[6] Buckman and Brady, *The nature and properties of soils*, The MacMillan Co., New York, 1960, p. 146.
[7] Pinck, Allison and Gaddy, *J. Am. Soc. Agron.*, 1948, **40**, 237.
[8] Stojanovic and Broadbent, *Soil Sci. Soc. Amer. Proc.*, 1956, **20**, 213.
[9] Lees and Porteous, *Plant and Soil*, 1950, **2**, 231.
[10] Lees, *ibid.*, 1948, **1**, 221.
[11] Lees, *Biochem. J.*, 1948, **42**, 531.
[12] Quastel and Scholefield, *Bacteriological Review*, 1951, **15**, 1.

CORRELATION BETWEEN THE TOTAL NITROGEN CONTENT AND THE CONTENT OF OTHER FORMS OF NITROGEN OBTAINED IN SOME SOUTH AFRICAN MAIZE SOILS

by

G. STIVEN

OPSOMMING

Dit is gevind dat die totale stikstofgehalte van die gronde in die mieliestreke van Suid-Afrika, nl. Transvaal en die Oranje-Vrystaat, goed met die minerale stikstof voor en na inkubasie in beide die boonste en onderste lae grond gekorreleer is.

Dit is vasgestel dat die hoeveelhede van minerale stikstof wat in hierdie gronde gevind is na inkubasie gewoonlik voorspel kan word indien die totale stikstof bekend is. Verder, as die toeganklike stikstof van die minerale stikstofgehalte in die grond bepaal kan word, dan kan dit ook van die totale stikstofgehalte afgelei word.

SUMMARY

The total nitrogen content of soils in the maize growing areas of the Transvaal and Orange Free State provinces of South Africa has been found to be well correlated with the content of mineral nitrogen (before and after incubation), as well as with the quantities of nitrate nitrogen produced during incubation.

It is concluded that the quantity of mineral nitrogen found in these soils after incubation could, in principle, be deduced from a knowledge of the total nitrogen content. Furthermore, if "available nitrogen" can be assessed from an estimate of the mineral nitrogen, it can alternatively be derived from the total nitrogen content.

INTRODUCTION

It was proposed long ago[1] that the study of a soil's capacity to produce nitrate nitrogen could provide reliable information of a soil's ability to supply nitrogen to a growing crop. In recent years great use has been made of this idea by many workers[2, 3, 4] as a means to developing a procedure whereby the quantity of available nitrogen could be estimated. However, it was also shown[5, 6, 7] that the quantities of nitrate produced by soils during various periods and conditions of incubation were well correlated with the quantities of total nitrogen found in those soils.

The incubation technique developed by the Iowa workers mentioned above has been used in our soil laboratories for studying the nitrifying capacities of soils taken from maize lands in the Transvaal and Orange Free State provinces of South Africa. In general, the quantities of nitrate nitrogen produced by these soils during incubation were small and often of the same order of magnitude as the error of a single determination. In some instances there was a decline in the nitrate nitrogen content during incubation, but an increase in the quantity of ammonia nitrogen, a result which is contrary to that experienced by workers in most countries where the quantities of ammonia nitrogen found after incubation are usually so small as to be unimportant. The limited nitrification in these soils indicated that the production of nitrate nitrogen during incubation was insufficient to furnish a general and reliable index of a soil's capacity to produce mineral nitrogen.

It is the purpose of this paper to show that in the soils of the maize-growing areas of the Transvaal and Orange Free State not only are the total nitrogen contents of these soils correlated with the contents of nitrate nitrogen found in these soils after incubation, but with several other forms of soil nitrogen as well.

EXPERIMENTAL

Soil samples were taken from 238 experimental sites situated on farms of the maize-growing areas of the Transvaal and Orange Free State provinces. Table I shows the number of soil samples taken in each of these provinces.

TABLE I

Distribution of the experimental sites

Area No.	Locality	No. of lands sampled
1	Western Transvaal	74
2	N.W. Free State	71
3	Eastern Transvaal Highveld	62
4	Eastern Orange Free State	31
Total		238

From each experimental site one composite soil sample was taken from the 0-6″ horizon, and one from the 6-12″ horizon, which are hereinafter referred to as the top- and subsoils respectively. The samples were taken in September, 1958, after the lands had been prepared for sowing, but prior to the planting of the crop, and analysed for nitrogen by the procedures outlined in Table II.

TABLE II

Methods of analysis

Procedure	Nitrogen determination in p.p.m.
1. Kjeldahl digestion	Total nitrogen
2. Extraction with a solution 0·05N with respect to H_2SO_4 and 0·50M with respect to K_2SO_4 on receipt of the sample	NH_3-N pre-incubation NO_3-N pre-incubation
3. Incubation for two weeks at 35°C at field capacity moisture, followed by extraction with the above solution	NH_3-N post-incubation NO_3-N post-incubation

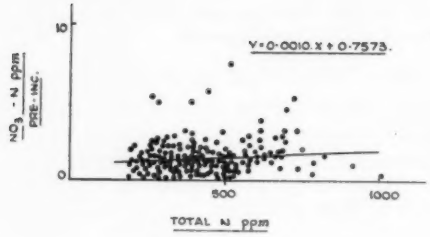

FIG. 1

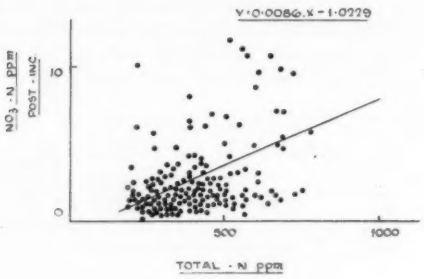

FIG. 2

FIG. 1—Relationship between total nitrogen and NO_3-N (pre-inc) in the subsoil
FIG. 2—Relationship between total nitrogen and NO_3-N (post inc.) n the subsoil

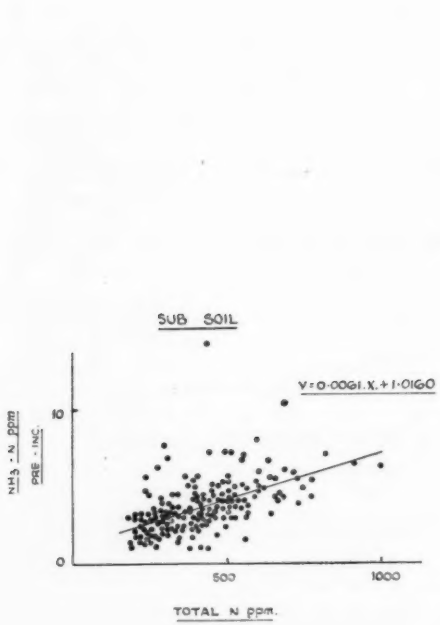

FIG. 3

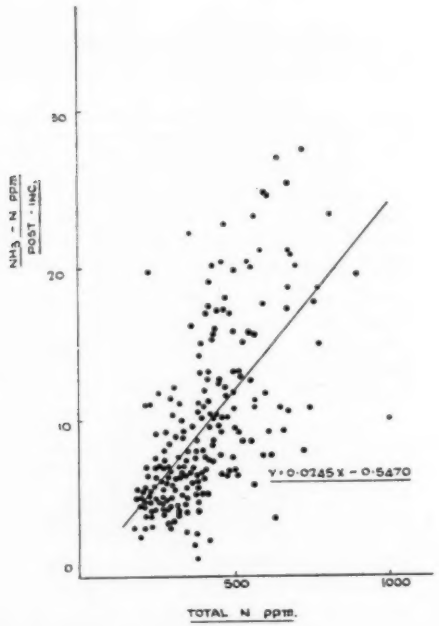

FIG. 4

FIG. 3—Relationship between total nitrogen and NH_3-N (pre-inc.) in the subsoil
FIG. 4—Relationship between total nitrogen and NH_3-N (post inc.) in the subsoil

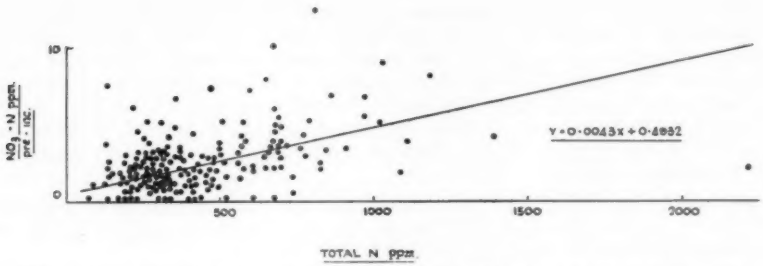

FIG. 5—Relationship between total nitrogen and NO_3-N (pre-inc.) in the topsoil

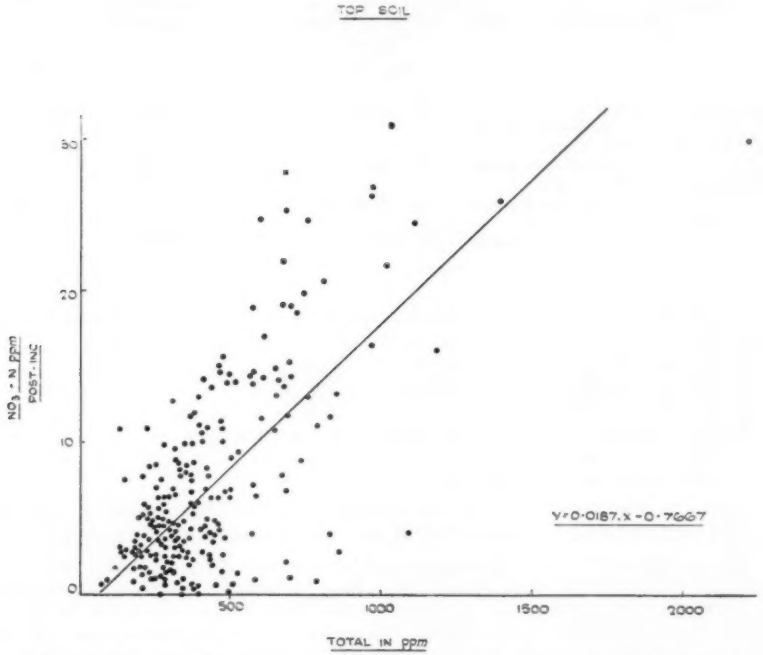

FIG. 6—Relationship between total nitrogen and NO_3-N (post-inc.) in the topsoil

CORRELATION AND REGRESSION ANALYSES

Correlation between the total nitrogen content and various forms of nitrogen in the subsoil. The quantities of total nitrogen found in the subsoils were found to be correlated with the following other forms of nitrogen in the subsoils:

(a) Nitrate nitrogen (pre-incubation) (Fig. 1)
(b) Nitrate nitrogen (post-incubation) (Fig. 2)
(c) Ammonia nitrogen (pre-incubation) (Fig. 3)
(d) Ammonia nitrogen (post-incubation) (Fig. 4)

The correlation of nitrate nitrogen (pre-incubation) with total nitrogen is poor, the coefficient of correlation is just significant ($0{\cdot}05>P>0{\cdot}01$). As the quantities of nitrate nitrogen (pre-incubation) are usually small, and often of the same order of magnitude as the error of a single determination, it is doubtful if the correlation has any value other than to show that such a correlation probably exists. The other correlation coefficients are all highly significant, so that in general, changes in the total nitrogen content will most probably be accompanied by relative changes in the other forms of nitrogen.

Regression equations have been calculated for each of the above correlations and they show quantitatively how changes in the total nitrogen status will affect the content of the other forms of nitrogen.

Table III summarises the results of these analyses.

TABLE III

Regression and correlation coefficients computed from the subsoil data

X	Y	Regression equation	r	P
Total nitrogen p.p.m.	NO_3-N pre-incubation p.p.m.	$Y=0{\cdot}0010\,X+0{\cdot}7573$	0·1500	$0{\cdot}05>P>0{\cdot}01$
Total nitrogen p.p.m.	NO_3-N post-incubation p.p.m.	$Y=0{\cdot}0086\,X-1{\cdot}0229$	0·4566	$<0{\cdot}001$
Total nitrogen p.p.m.	NH_3-N pre-incubation p.p.m.	$Y=0{\cdot}0061\,X+1{\cdot}0160$	0·6001	$<0{\cdot}001$
Total nitrogen p.p.m.	NH_3-N post-incubation p.p.m.	$Y=0{\cdot}0245\,X-0{\cdot}5470$	0·6382	$<0{\cdot}001$

Correlation between the total nitrogen content and various forms of nitrogen in the topsoil. The total nitrogen was found to be correlated with the following:

(e) Nitrate nitrogen (pre-incubation) (Fig. 5)
(f) Nitrate nitrogen (post-incubation) (Fig. 6)
(g) Ammonia nitrogen (pre-incubation) (Fig. 7)
(h) The change in the nitrate nitrogen content expressed as:—
(NO_3-N post-inc.)
(NO_3-N pre-inc.)$= \Delta\ NO_3$-N (Fig. 8)

The total nitrogen content in the topsoil unlike that in the subsoil, was found to be uncorrelated with the quantities of post-incubation ammonia nitrogen (Fig. 9).

The coefficients of the abovementioned correlations were all found to be highly significant. The regression equations offer some idea of the quantitative changes to be expected in these forms of nitrogen when changes have occurred in the total nitrogen content. Although, in general, the above correlations are apparently better than those calculated for the subsoils, the scatter of the experimental points is still wide so that the regression formulae should be considered only as information and not as established prediction devices.

Table IV summarises the results of these analyses.

Correlation between the total nitrogen and the mineral nitrogen contents in the topsoil. For reasons already stated in the introduction, the quantities of post-incubation nitrate nitrogen in these soils could not provide a sufficiently general index of the capacity of these soils to produce mineral nitrogen during incubation. As the sum of the quantities of post-incubation ammonia nitrogen and nitrate nitrogen was always greater than that of the corresponding pre-incubation values, it was

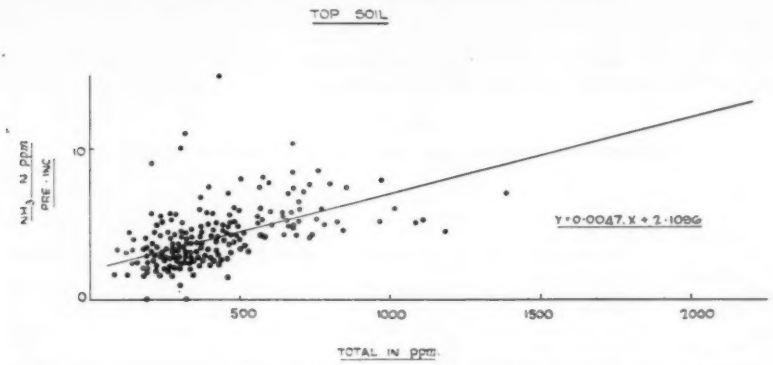

FIG. 7—Relationship between total nitrogen and NH_3-N (pre-inc.) in the topsoil

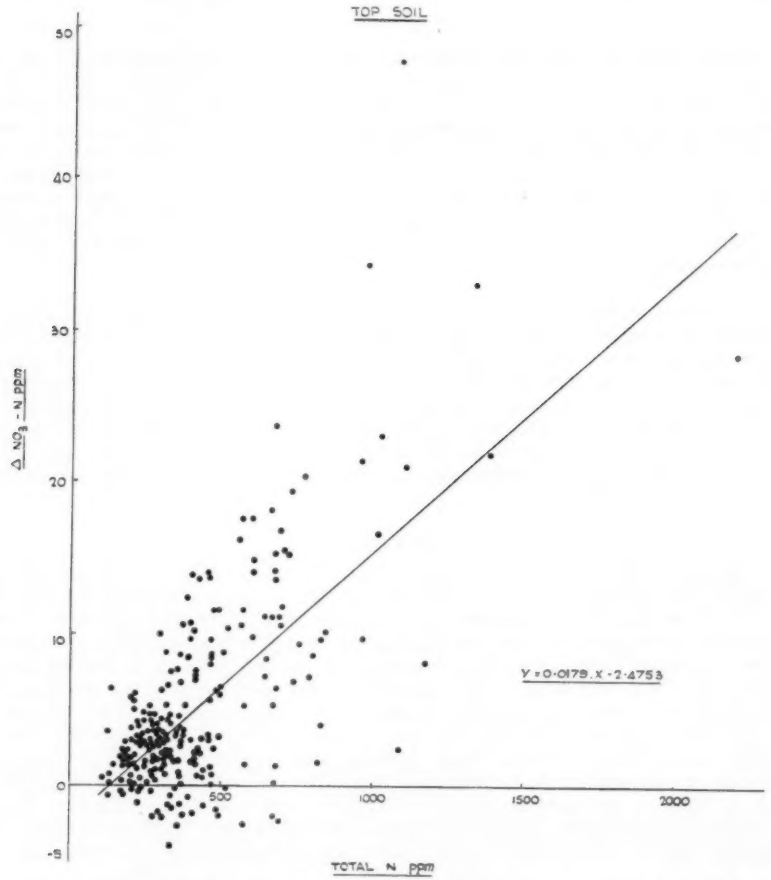

FIG. 8—Relationship between total nitrogen and the change in NO_3-N during incubation (ΔNO_3-N) in the topsoil

assumed that the former would more realistically represent the capacity of these soils to produce mineral nitrogen during incubation.

TABLE IV

Regression and correlation coefficients computed from the topsoil data

X	Y	Regression Formula	r	P
Total nitrogen p.p.m.	NO_3-N pre-incubation p.p.m.	Y=0·0043 X+0·4932	0·5003	<0·001
Total nitrogen p.p.m.	NO_3N post-incubation p.p.m.	Y=0·0187 X−0·7667	0·6418	<0·001
Total nitrogen p.p.m.	NH_3-N pre-incubation p.p.m.	Y=0·0047 X+2·1096	0·6147	<0·001
Total nitrogen p.p.m.	NH_3-N post-incubation p.p.m.	Y=0·0003 X+4·8196	0·0205	N.S.
Total nitrogen p.p.m.	Δ NO_3-N	Y=0·0179 X−2.4757	0·6885	<0·001

The total nitrogen content was found to be significantly correlated with the sum of the post-incubation ammonia and nitrate nitrogen (hereinafter referred to as mineral nitrogen). The coefficient of correlation ($r=O·7576$, $P<O·001$) was found to be highly significant. The experimental points (Fig. 10) appear to be well represented by the regression line, so that the regression equation could probably be used for predicting the quantitative change in mineral nitrogen, given the corresponding change in the total nitrogen, if further statistical examinations of the parameters were carried out. The scatter in the experimental points suggests that if some of the errors with which the incubation technique is plagued could be removed, a better correlation might result.

Correlation between the change in the nitrate nitrogen content and the change in the ammonia nitrogen content during incubation. The change in the nitrate nitrogen content during incubation expressed as the difference between the pre- and post-incubation quantities, and denoted by Δ NO_3-N, was found to be well correlated with the change in the ammonia nitrogen content, similarly calculated, during incubation, and denoted by Δ NH_3-N (Fig. 11). The coefficient of correlation ($r=-0·5351$) was found to be highly significant ($P<O·001$). The regression equations shown in Fig. 11 offer approximate quantitative expressions for estimating relative changes in either of the variables.

DISCUSSION

Previous workers[1, 2, 4] have assumed that the measurement of nitrate nitrogen or mineral nitrogen produced by a soil during incubation will provide the best and most direct estimate of the quantity of nitrogen which would be available to a crop. Waksman[1] mentioned several investigators who had obtained good correlations between nitrifying power and crop production, but at the same time he also mentioned the work of others who were unable to establish any such correlations. He dismissed their results by saying that a faulty incubation technique had been used, but he did not indicate where the fault lay. Saunders[4] mentioned that whilst he was able to establish relationships between total nitrogen and crop production, he thought a "more direct procedure is to be preferred" for estimating the release of soil nitrogen to crops, and he assumed that the technique[2], with small modifications, would provide this information. Fitts *et al.*[2] seemed to think that "from theoretical considerations and of its similarity to soil processes, there are reasons to believe . . ."

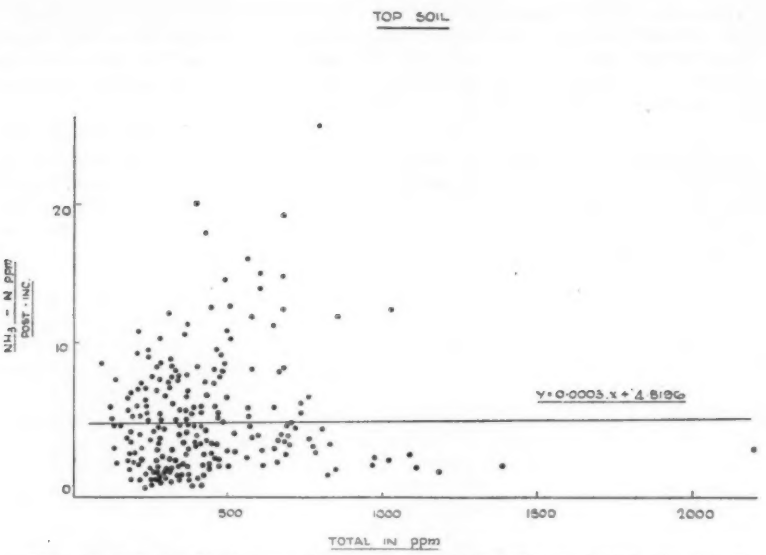

FIG. 9—Relationship between total nitrogen and NH_3N (post-inc.) in the topsoil

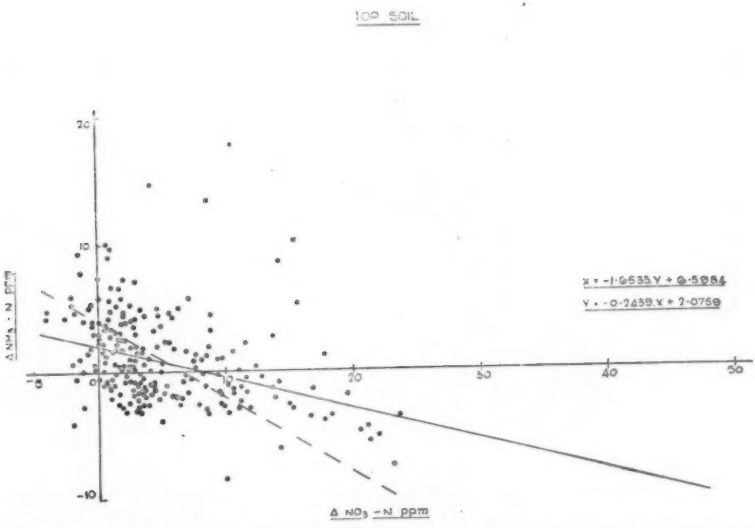

FIG. 11—Relationship between the changes in the NO_3-N content during incubation (ΔNO_3-N) and the corresponding changes in the NH_3-N content (ΔNH_3-N) in the topsoil

that the amounts of nitrogen produced during incubation by biological mineralisation would be the best estimate of a soil's ability to provide nitrogen to a crop. It seems that they did not attempt to investigate if nitrogen measured by any other means would serve this purpose, or if correlations existed between biologically mineralised nitrogen and other forms of nitrogen in their soils.

The parameters of the regression equations shown in this paper would need to be examined further to discover their physical import before the equations could be used as prediction devices. There appears to be no point in doing this until the errors inherent in the incubation technique can be resolved, and the usefulness of the biologically mineralised nitrogen as a measure of available nitrogen has been established more firmly.

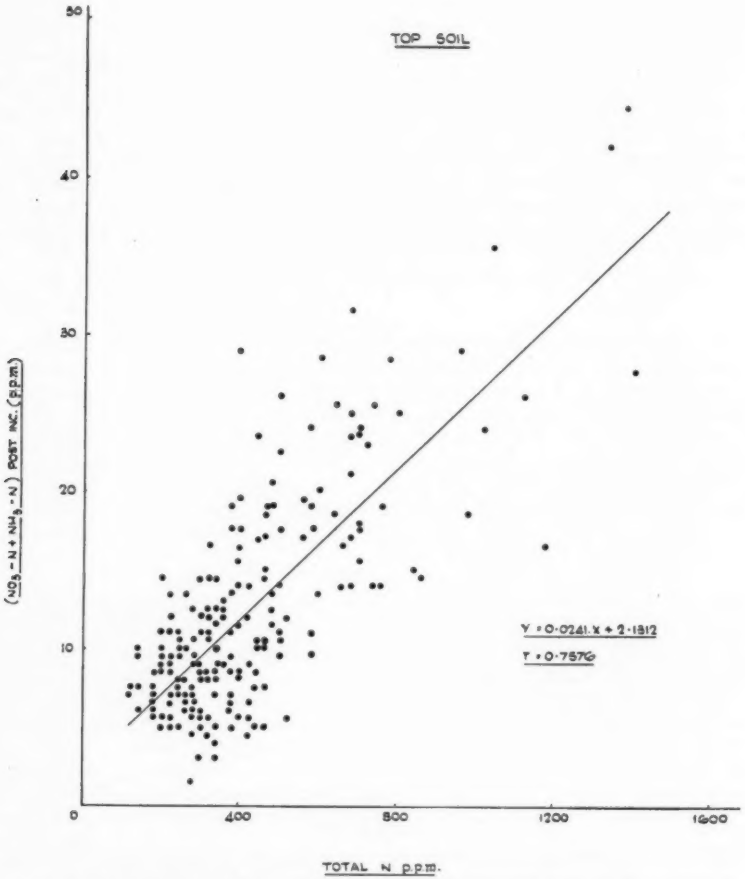

FIG. 10—Relationship between total nitrogen and mineral nitrogen in the topsoil

The fact that these various measures of soil nitrogen can be shown to be correlated with the total nitrogen makes the task of deriving a yield prediction function based on the soil nitrogen status more difficult, for if one of these measures of soil nitrogen can be shown to be correlated with crop yield, in the manner that Mackenzie, Marr and Stiven[7] have shown for soil phosphate, then all the remainder should be similarly correlated.

The disadvantages of the incubation technique have been well presented by Harmsen and van Schreven,[9] and more recently it has been suggested[10] that it might well be replaced by a technique whereby soils are incubated with N NaOH for 40-42 hours.

CONCLUSION

From a knowledge of the total nitrogen status of soils in the maize growing areas of the Transvaal and Orange Free State approximate quantitative expressions may be obtained for several other forms of nitrogen, in particular the quantities of nitrate nitrogen produced during incubation, and the quantities of mineral nitrogen found in these soils after incubation.

Because total nitrogen can be measured more directly than mineral nitrogen, which is generally used for predicting crop needs for nitrogen, it is concluded that at least equally good predictions could be made from a knowledge of the total nitrogen content of these soils.

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REFERENCES

[1] Waksman, *Soil Sci.*, 1923, **16**, 55.
[2] Fitts, Bartholomew and Heidel, *Soil Sci. Soc. Amer. Proc.*, 1955, **19**, 69.
[3] Hanway and Dumenil, *ibid.*, 1955, **19**, 77.
[4] Saunder, Ellis and Hall, *J. Soil Sci.*, 1957, **8**, 301.
[5] Fraps and Sterges, *Texas Agric. Expt. Sta. Bull.*, 1947, No. 693, 1.
[6] Gainey, *Soil Sci.*, 1936, **42**, 157.
[7] Allison and Sterling, *ibid.*, 1949, **67**, 239.
[8] Mackenzie, Marr and Stiven, *J. S. African Chem. Inst.*, 1958, **11**, 69.
[9] Harmsen and van Schreven, *Advances in Agronomy*, Vol. VII, Academic Press, New York, p. 299.
[10] Cornfield, *Nature*, 1960, **187**, 260.

PREPARATIVE LINEAR CHROMATOGRAPHY: THE EQUATION OF THE ELUTION CURVES AND THE RECOVERY OF THE SOLUTES

by

P. C. HAARHOFF, P. C. VAN BERGE AND VICTOR PRETORIUS

OPSOMMING

'n Vergelyking, wat die verandering in eksperimentele toestande oor die kolomlengte in ag neem, is afgelei vir die eluasiekromme van 'n opgeloste stof met eindige inlaatvolume. Die wyse waarop die eluaat van 'n tweekomponentmengsel verdeel moet word om stowwe met 'n verlangde suiwerheid te lewer, is bespreek in terme van die opgeloste stof-bandoorvleueling.

SUMMARY

An equation is derived for the elution curve of a solute of finite inlet volume, which takes the variation of experimental conditions along the column length into account. The way in which the eluate of a two component mixture should be divided to yield solute fractions of a desired purity is discussed in terms of the solute band overlap.

It has recently been shown[1] that the yields which may be obtained by means of preparative chromatography of a two component mixture may be greatly increased by dividing the eluate into three, and not into two fractions, and discarding the middle fraction. The present paper is concerned with a description of the way in which the division of the eluate should be carried out to provide fractions of a desired purity. The investigation is based on a consideration of the overlap of the elution curves of the two solutes.

THE EQUATION OF THE ELUTION CURVE FOR FINITE SAMPLE VOLUMES AT THE COLUMN INLET

In linear non-ideal chromatography the elution curve of a single solute of infinitesimally small volume at the column inlet is a probability function.[2] The concentration of the solute in the mobile phase at the column outlet is given by*

$$c\ (V) = \frac{M}{\sqrt{2\,\pi\,\sigma^2}} \exp\left\{-\frac{(V - \overline{V} - V^1)^2}{2\sigma^2}\right\} \quad .. \quad .. \quad .. \quad (1)$$

when the solute is introduced into the column at $V = V^1$. The constant M is equal to the number of moles of the solute, since

$$\int_{-\infty}^{+\infty} c(V)\, d\, V = M \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

*The symbols are defined at the end of the paper.

Equation 1 has been extended by Glueckauf[3], van Deemter *et al.*[4] and by Porter *et al.*[5] to include the case where the volume of the sample at the column inlet is not negligible. These authors have assumed that the experimental conditions do not vary over the length of the column. This assumption is not valid in gas chromatography, where linear velocity of the eluent varies continuously along the column length as a result of the compressibility of the carrier gas.[6, 7, 8] The treatments mentioned above will now be extended to take into account any variation of operating conditions along the column length. A general consideration of the conservation of mass will be given, which does not in any way depend on the plate theory as do the expressions derived by the above authors.

Consider a solute of finite inlet volume W which is introduced into the mobile phase. Other methods of sample introduction, e.g. deposition of the solutes on the stationary phase,[3] may be taken into account by defining the sample inlet volume as the volume of the eluent which is introduced into the column while the solute concentration in the mobile phase at the column inlet, C, is finite. C may be regarded as a function of the volume of the eluent introduced from the start of injection of the sample, V_i. The number of moles, dM, in the portion of the solute which enters the column while V_i varies from $V_i = V_i^1$ to $V_i = V_i^1 + dV_i^1$, is given by

$$dM = C(V_i^1)dV_i^1 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Since $V = Fv_0t$ .. .. (4)

and $V_i = Fv_it$ .. .. (5)

it follows that $V_i = \frac{v_i}{v_0} V$ .. .. (6)

The contribution of the abovementioned solute portion to the elution curve is consequently given, from equations (1), (3) and (6), by

$$dc = \frac{Cv_i}{v_0} \cdot \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left\{\frac{-(V - \overline{V} - V^1)^2}{2\sigma^2}\right\} dV^1 \quad .. \quad .. \quad (7)$$

During the interval in which solute is introduced into the column, V varies from $V = O$ to $V = W^0$, where

$$W^0 = \frac{v_0}{v_i} W \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

as may be seen from equation (6). Let

$$C^0 = \frac{v_i}{v_0} C \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (9)$$

The elution curve may be obtained by integration of equation (7). By using equations (8) and (9), we obtain

$$c(V) = \int_O^{W^0} \frac{C^0}{\sqrt{2\pi\sigma^2}} \exp\left\{\frac{-(V - \overline{V} - V^1)^2}{2\sigma^2}\right\} dV^1 \quad .. \quad .. \quad (10)$$

Before equation (10) can be integrated C^0, and thus C, must be expressed as a function of V or V^1. The form of such a function will depend on the type of inlet

system.[5, 9] For the present purpose, however, it will be assumed that C(and hence C^0) remains constant during the introduction of the sample, as appears to be a good approximation in practice.[9]

If the experimental conditions do not vary along the length of the column, equation (10) may be shown to reduce to the corresponding expressions given by Glueckauf,[3] van Deemter *et al.*[4] and by Porter *et al.*[5]

In general, W^0 may be regarded as the volume which would have been occupied by the solute had it been transported from the inlet to the outlet without any disturbing influences, such as diffusion (i.e. if $\sigma = 0$). Since it follows from equations (3), (8) and (9) that

$$M = WC = W^0C^0 \qquad (11)$$

C^0 may be similarly interpreted. The terms "equivalent inlet volume" and "equivalent inlet concentration" for W^0 and C^0 respectively suggest themselves.

In gas chromatography the linear velocity of the gaseous phase is inversely proportional to the gas pressure and W^0 and C^0 may be written, by using equations (8) and (9), as

$$W^0 = \frac{P_i}{P_0} W \qquad (12)$$

and

$$C^0 = \frac{P_0}{P_i} C \qquad (13)$$

Equations (12) and (13) show that dilution of the solute plug, in addition to the dilution due to disturbing influences, is caused by the decompression of the carrier gas along the column length. Hence, the solute plug may be visualised as expanding with the carrier gas in a similar fashion to that discussed by Martin[6] and by Giddings *et al.*[7]

The manner in which this phenomenon will manifest itself in practice may be considered as follows. Elution curves corresponding to finite inlet volumes are best studied by means of a comparison with those for which the inlet volumes are negligible, i.e. by measuring the eluate volume from the centre of mass of the solute band, in units of σ. This procedure has been followed in Fig. 1, where the influence of the pressure ratio P_i/P_0 on a gas chromatographic elution curve has been depicted. For all calculations, C has been taken as a constant, and the sample inlet volume W as 4σ, i.e. as a constant multiple of the band width of a solute for which $W = 0$. Equations (12), (13), (14), (16), (29) and (49) have been used for this purpose. The results in Fig. 1 show clearly that the pressure drop along the column causes a dilution of the solute bands and that the shapes of the elution curves are also changed. A dilution of solute bands was experimentally observed by de Wet and Pretorius,[9] and was ascribed to the sample inlet system. It is now evident that this dilution was partly caused by the effects discussed above.

THE SEPARATION OF THE ELUATE INTO FRACTIONS

Consider a mixture of two solutes, I and II, which are eluted in that order, and which are separated by means of the three fraction technique. The volumes at which

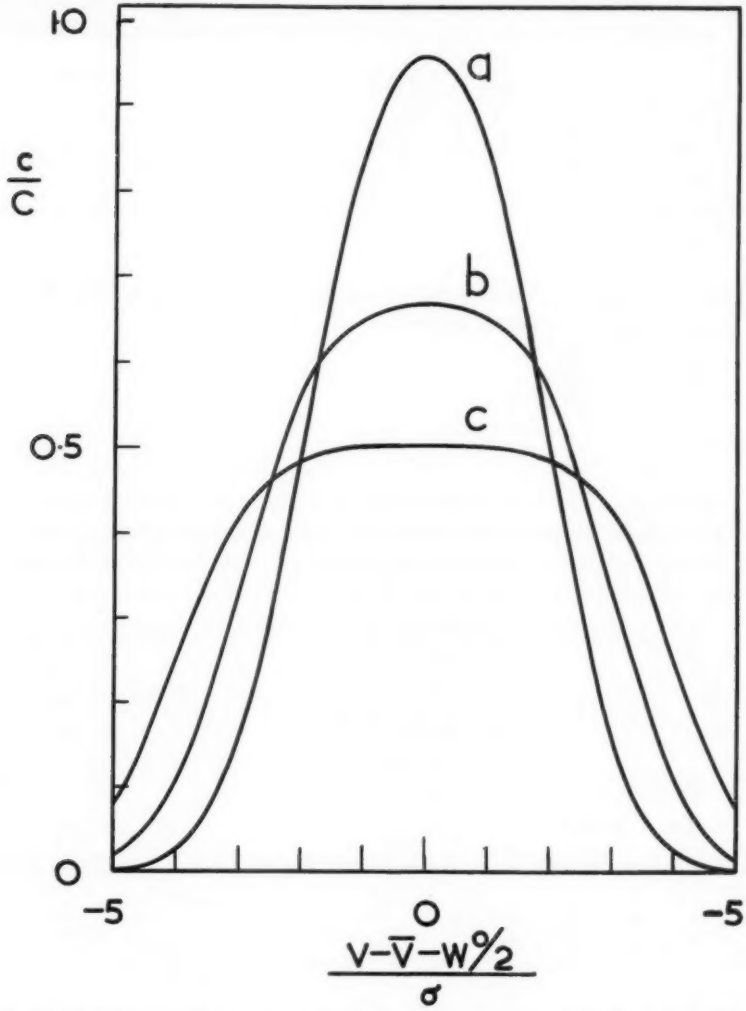

FIG. 1—The influence of the pressure ratio on a gas chromatographic elution curve. The ratio of the solute concentration at the column outlet to the inlet concentration has been plotted against the eluate volume as measured from the centre of mass of the solute band, in units of σ, for $W = 4\sigma$ and (a), $P_i/P_0 = 1$, (b), $P_i/P_0 = 1{\cdot}5$, (c), $P_i/P_0 = 2$.

the eluate should be cut may best be found by expressing all volumes in units of σ_1, and by measuring the volume of the eluate from the centre of mass of the solute I. Let

$$x = \frac{V - \overline{V}_I - W^0/2}{\sigma_I} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (14)$$

$$y = \frac{V^1 - W^0/2}{\sigma_I} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (15)$$

$$w = \frac{W^0}{\sigma_I} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (16)$$

The overall efficiency with which the solutes are separated may be described[10] by the peak resolution, R, which is defined as

$$R = \frac{\overline{V}_{II} - \overline{V}_I}{\sigma_I + \sigma_{II}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (17)$$

Let

$$q = \frac{\sigma_{II}}{\sigma_I} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (18)$$

represent the ratio of the band widths of the solutes, and let m be the total number of moles of a solute in a volume V of the eluate, which is measured from the instant at which solute is first introduced into the column. By means of equations (14)-(18), equations (10) and (11) may be written for each solute as

$$\frac{dm_I}{dx} = c_I \sigma_I = C_I^0 \sigma_I \int_{-w/2}^{+w/2} \frac{1}{\sqrt{2\pi}} \exp\left\{-\frac{(x-y)^2}{2}\right\} dy \quad .. \quad .. \quad (19)$$

$$\frac{dm_{II}}{dx} = c_{II} \sigma_I = C_{II}^0 \sigma_I \int_{-w/2}^{+w/2} \frac{1}{\sqrt{2\pi q^2}} \exp\left\{-\frac{[x-y-R(1+q)]^2}{2q^2}\right\} dy \quad (20)$$

and

$$M_I = C_I^0 \sigma_I w \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (21)$$

$$M_{II} = C_{II}^0 \sigma_I w \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (22)$$

If a cut is made at $V = V^c$, the corresponding value of x is given by equation (14) as

$$x^c = \frac{V^c - \overline{V}_I - W^0/2}{\sigma_I} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (23)$$

Let

$$\Delta M_I = \int_{V^c}^{\infty} c_I(V) dV \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (24)$$

and

$$\Delta M_{II} = \int_{-\infty}^{V^c} c_{II}(V) dV \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (25)$$

be defined as the minor portions of the solutes I and II. From equations (19), (20) and (23)-(25), ΔM_I and ΔM_{II} are given by

$$\Delta M_I = C_I^0 \sigma_I \int_{x^c}^{\infty} dx \int_{-w/2}^{+w/2} \frac{1}{\sqrt{2\pi}} \exp\left\{-\frac{(x-y)^2}{2}\right\} dy \quad .. \quad .. \quad (26)$$

$$\Delta M_{II} = C_{II}^0 \sigma_I \int_{-\infty}^{x^c} dx \int_{-w/2}^{+w/2} \frac{1}{\sqrt{2\pi q^2}} \exp\left\{-\frac{[x-y-R(1+q)]^2}{2q^2}\right\} dy \quad (27)$$

These integrals may be evaluated by means of the second integral of the probability function. Let

$$Z(X) = \frac{e^{-X^2/2}}{\sqrt{2\pi}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (28)$$

$$P_e(X) = \int_{-\infty}^{X} Z(X^1) dX^1 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (29)$$

$$Y(X) = \int_{-\infty}^{X} P_e(X^1) dX^1 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (30)$$

denote the probability function and its successive integrals. It may be verified by differentiation of equations (30) and (31) that

$$Y(X) = X\,P_e(X) + Z(X) \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (31)$$

The following relations may be obtained from equations (28)-(31):

$$Z(-X) = Z(X) \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (32)$$

$$P_e(-X) = 1 - P_e(X) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (33)$$

$$Y(-X) = Y(X) - X \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (34)$$

Equations (26) and (27) may now be integrated.

Let

$$x^1 = y - x \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (35)$$

From equations (26), (28), (29), (33) and (35),

$$\Delta M_I = C_I^0 \sigma_I \int_{-w/2}^{+w/2} [1 - P_e(x^c - y)]\, dy \quad .. \quad .. \quad .. \quad .. \quad .. \quad (36)$$

Let

$$y^1 = x^c - y \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (37)$$

From equations (30), (36) and (37),

$$\Delta M_I = C_I^0 \sigma_I [w - Y(x^c + w/2) + Y(x^c - w/2)] \quad .. \quad .. \quad .. \quad (38)$$

Similarly, it may be shown from equations (27)-(34) that

$$\Delta M_{II} = C_{II}^0 \sigma_I \left[w - qY\left(\frac{R(1+q) - x^c + w/2}{q}\right) + qY\left(\frac{R(1+q) - x^c - w/2}{q}\right)\right] \quad (39)$$

These results may be more simply expressed in terms of the major fractional portion of each solute,

$$\theta = \frac{M - \Delta M}{M} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (40)$$

By using equations (21), (22), (38), (39) and (40),

$$\theta_I = \frac{Y\,(x^c + w/2) - Y\,(x^c - w/2)}{w} \quad .. \quad .. \quad .. \quad .. \quad (41)$$

$$\theta_{II} = \frac{q}{w}\left[Y\left(\frac{R(1+q) - x^c + w/2}{q}\right) - Y\left(\frac{R(1+q) - x^c - w/2}{q}\right)\right] \quad (42)$$

These equations may be used to find the fractional portions into which each solute is divided at a given cut of the eluate.

To facilitate the calculations a table of values of Y(X) may be set up by means of equation (31) and already existing tables of values[11] of Z(X) and $P_e(X)$. Positive values only of X need be considered (equation (34)).

If a maximum impurity ratio

$$\eta_I = \frac{\Delta M_{II}}{M_I - \Delta M_I} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (43)$$

is allowed in the portion of the solute I which is recovered from the eluate, the volume at which this fraction should be cut from the rest of the eluate, and the amount of the solute I which is recovered, may be determined as follows.

From equations (40) and (43),

$$\eta_I = \frac{M_{II}}{M_I}\left(\frac{1 - \theta_{II}}{\theta_I}\right) \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (44)$$

Let

$$\eta_I^0 = \frac{M_I\,\eta_I}{M_{II}} = \frac{1 - \theta_{II}}{\theta_I} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (45)$$

η_I^0 may be termed the equivalent impurity ratio of the solute I for an equimolar mixture. The value of x^c corresponding to η_I^0, x_I^c, may ae numerically determined from equations (41), (42) and (45) for each set of values of R, q, w and η_I^0. The volume at which the solute I is to be cut from the eluate, V_I^c, is given by equation (23). The amount of the solute I which may be recovered is given, from equation (40), by

$$[M_r]_I = M_I - \Delta M_I = \theta_I M_I \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (46)$$

and the amount of the solute II, which is present as an impurity, is given by

$$\Delta M_{II} = (1 - \theta_{II})M_{II} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (47)$$

The volume, V_{II}^c, at which the solute II must be cut from the eluate and the amount of the solute II which may be recovered, are found by calculating the value, x_{II}^c, of x^c corresponding to

$$\eta_{II}^0 = \frac{M_{II}\,\eta_{II}}{M_I} = \frac{1 - \theta_I}{\theta_{II}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (48)$$

from equations (41), (42) and (48). Alternatively, a mirror image of the chromatogram about the midpoint between the two peaks may be considered. The subscript I then

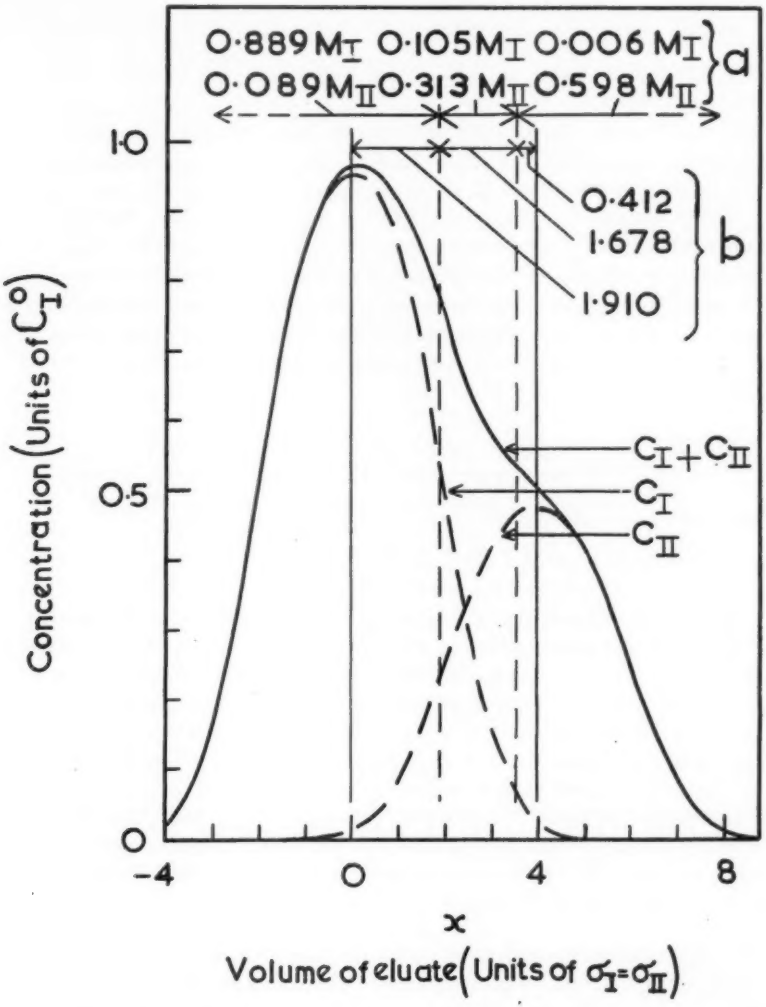

FIG. 2—Division of the eluate into portions for R = 2, q = 1, w = 4, $M_I/M_{II} = 2$, $\eta_I = 0{\cdot}05$ and $\eta_{II} = 0{\cdot}02$. (a) Division of solutes. (b) Division of volume. Dashed vertical lines indicate cut volumes. Full vertical lines indicate peak maxima.

applies to the second solute. The value of x_I^c thus obtained, denotes the volume at which the solute II should be separated from the eluate, V_{II}^c, measured in units of σ_{II}, from the centre of mass ($\overline{V}_{II} + W^0/2$) of the solute II, in the direction of the centre of mass of the solute I.

Two cut volumes, V_I^c and V_{II}^c, are obtained by the above methods for each chromatogram. In general, V_{II}^c will be larger than V_I^c, and the calculated amount of each solute may be recovered. If, however, the chosen values of η_I and η_{II} are too large, it may happen that V_{II}^c is smaller than V_I^c, in which case the cuts have no practical significance if both solutes are to be recovered during the same run. This difficulty may be avoided by choosing sufficiently small values of η_I and η_{II}.

An illustration of the foregoing discussion is given in Fig. 2. The chromatogram of a solute mixture of two components has been calculated using the values $R = 2$, $q = 1$, $w = 4$ and $M_I = 2M_{II}$, which may well occur in practice. From equations (19), (20), (28) and (29),

$$c_I = C_I^0 [P_e (x + w/2) - P_e (x - w/2)] \quad .. \quad .. \quad .. \quad .. \quad (49)$$

$$c_{II} = C_{II}^0 \left[P_e\left(\frac{x + w/2 - R(1+q)}{q}\right) - P_e\left(\frac{x - w/2 - R(1+q)}{q}\right)\right] \quad (50)$$

By substituting the values of R, q, w and M_I/M_{II}, one obtains

$$c_I = C_{II}^0 [P_e(x + 2) - P_e(x - 2)] \quad .. \quad .. \quad .. \quad .. \quad .. \quad (51)$$

$$c_{II} = 0{\cdot}5\, C_I^0 [P_e(x - 2) - P_e(x - 6)] \quad .. \quad .. \quad .. \quad .. \quad .. \quad (52)$$

Let it be assumed that the first solute must be recovered with an impurity ratio $\eta_e = 0{\cdot}05$, and the second with $\eta_{II} = 0{\cdot}02$. For the first solute, x_I^c is found from equations (41), (42) and (45) as $x_I^c = 1{\cdot}910$, and the recovered portion is $0{\cdot}889M_1$ (equations (41) and (46)). By considering a mirror image of the chromatogram about the midpoint between the peaks, a value $x_I^c = 0{\cdot}412$ is obtained from equations (41), (42) and (45). Consequently, $x_{II}^c = 3{\cdot}588$. The recovered portion is $0{\cdot}598\ M_{II}$. It may be seen that the equivalent inlet volume of the sample, $W^0 = 4\sigma_I$, is equal to the volume between the peak maxima. The extent to which band broadening occurs as a result of the equivalent inlet volume may be estimated by noting that the width of a peak for which $W^0 = O$, is $4\sigma_I$, as measured by the volume between the intercepts on the base line of the tangents at the inflection points to the elution curve.

We are indebted to the South African Council for Scientific and Industrial Research and to African Explosives and Chemical Industries, Ltd., for financial assistance, to the latter for allowing one of us (P. C. v. B.) to participate in this project, and to the South African Atomic Energy Board for the award of a bursary to P. C. H. We should also like to thank Prof. F. L. Clark for the stimulating interest he has shown in this work.

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LIST OF SYMBOLS

I	subscript designating solute which is eluted first
II	subscript designating solute which is eluted last
C	solute concentration in mobile phase at column inlet
C^0	equivalent inlet solute concentration
c	solute concentration in mobile phase at column outlet
F	cross-sectional area of column
M	number of moles of solute introduced per sample
M_r	number of moles of solute recovered per sample
ΔM	minor portion of solute
m	number of moles of solute which have passed through column outlet
P_e	first integral of probability function
P_i	pressure of carrier gas at column inlet
P_0	pressure of carrier gas at column outlet
q	ratio of band width of solute II to band width of solute I (for infinitesimally small samples)
R	peak resolution (for infinitesimally small samples)
t	time measured from start of injection of finite sample volume
V	volume of eluate measured at column outlet from t = O.
$\overline{V}$	retention volume of solute
V^1	value of V at which a portion of sample is introduced into column
V^c	value of V at which a solute portion is cut from the eluate
V_i	volume of eluent measured at column inlet from t = O.
V_i^1	value of V_i at which a portion of sample is introduced into column
v_i	linear velocity of mobile phase at column inlet
v_0	linear velocity of mobile phase at column outlet
W	sample volume at column inlet
W^0	equivalent inlet volume of sample
w	value of W^0 in units of σ_I
x	volume of eluate, measured at column outlet, from the centre of mass of solute I, in units of σ_I
x^c	value of x corresponding to V^c
x^1	convenient parameter
Y	second integral of probability function
y, y^1	convenient parameters
Z	probability function
θ	major fractional portion of solute
η	impurity ratio of recovered solute portion
η^0	equivalent impurity ratio for equimolar mixture
σ	standard deviation of solute band (for infinitesimally small samples)

REFERENCES

[1] Haarhoff, van Berge and Pretorius, *Trans. Faraday Soc.*, in the press.
[2] Klinkenberg and Sjenitzer, *Chem. Eng. Sci.*, 1956, **5**, 258.
[3] Glueckauf, *Trans. Faraday Soc.*, 1955, **51**, 34.
[4] van Deemter, Zuiderweg and Klinkenberg, *Chem. Eng. Sci.*, 1956, **5**, 271.
[5] Porter, Deal and Stross, *J. Am. Chem. Soc.*, 1956, **78**, 2999.
[6] Martin, *Vapour Phase Chromatography*, edited by Desty, Butterworths Scientific Publications, London, 1957, p. 4.
[7] Stewart, Seager and Giddings, *Anal. Chem.*, 1959, **31**, 1738.
[8] Haarhoff and Pretorius, *J.S. African Chem. Inst.*, 1960, **13**, 97.
[9] de Wet and Pretorius, *Anal. Chem.*, 1960, **32**, 169.
[10] Haarhoff and Pretorius, *J.S. African Chem. Inst.*, 1961, **14**, 22.
[11] *Biometrika Tables for Statisticians*, Vol. 1, 2nd edition, edited by Pearson and Hartley, Cambridge University Press, Cambridge, 1958, p. 104.

C.S.I.R. R.E. 65

RADIOLYSIS OF FERROUS SULPHATE SOLUTIONS WITH STANDARDISED INTERNAL SOURCES OF POLONIUM-210

by

J. STEYN and D. VAN AS

OPSOMMING

Die radiolitiese opbrengs van ferri-ione in beide ontlugde en lugversadigde „Fricke"-dosismeteroplossings is bepaal met bestraling deur interne bronne van die α-straler polonium-210. Die radioaktiwiteit is gestandaardiseer met 4π-vloeistofsintillasietelling. Die volgende G-waardes is gevind:

Lugversadigde oplossing: $5{\cdot}08 \pm 0{\cdot}02$
Ontlugde oplossing: $3{\cdot}53 \pm 0{\cdot}01$

SUMMARY

The radiolytic yield of ferric ions in both aerated and deaerated "Fricke" dosimeter solutions was determined for irradiation with internal sources of the α-emitter polonium-210. The radioactivity was standardized by 4π-liquid scintillating counting. The following G-values were found:

Aerated solution: $5{\cdot}08 \pm 0{\cdot}02$
Deaerated solution: $3{\cdot}53 \pm 0{\cdot}01$

INTRODUCTION

A characteristic phenomenon in the radiolysis of aqueous solutions is the variation of yields with the type of radiation. Accurate determinations of G-values (the yield in ions per 100 eV) over a wide range of radiation qualities are thus necessary, not only for a complete calibration of chemical dosimeter systems, but also in assisting the further development of theory.[1] Irradiation with alpha-particles plays an important part in establishing the overall picture, hence a number of publications have already appeared describing irradiation experiments with beams of accelerated helium ions[2] as well as radioactive sources of α-particles. In this respect the α-emitter polonium-210 forms a convenient and readily available radiation source.

Regarding Po-210 irradiation of the air-saturated ferrous sulphate solution known as the "Fricke" dosimeter, G-values have been published ranging from 6·3 to 5·1·[3, 4, 5, 6, 7] with a tendency towards the lower values as time progressed. It is noteworthy that the latest values, 5·5 and 5·1, published simultaneously by Lefort and Tarrago,[6] and Trumbore and Hart[7] respectively, are in disagreement. Obviously, an accurate value of G at this particular value of linear energy transfer (or rate of energy loss along the track) has not yet been established.

The present publication describes determinations of G for both aerated and deaerated Fricke systems with carrier-free polonium-210, dissolved in the dosimeter solution, acting as internal radiation source. The radiation energy absorbed by the system was determined from an accurate standardisation of the amount of radioactive material present in the dosimeter solutions.

EXPERIMENTAL

Method. The equipment and method were substantially the same as have been used in earlier work[8] with phosphorous-32. The increasing ferric ion concentration was determined spectrophotometrically over a period of about eight to twelve days,

during which time small samples of the dosimeter solution were removed from the irradiation flasks and diluted for the radioactivity measurements. G is then found from the following expression: (see equation (3), reference 8).

$$Dt = \frac{k.G.A_0.E}{N.\lambda}(1 - e^{-\lambda t}) \times 10^7 \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

$$\text{or } G = \frac{H.N.\lambda}{A_0.k.E.} \times 10^{-7} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where H is the slope of the straight line obtained when Dt, the optical density of the irradiated solutions, is plotted against $(1-e^{-\lambda t})$. Here λ is the decay constant of polonium-210, k is the molar extinction coefficient of the ferric solution, E is the energy of the α-particles in Mev., A_0 is the specific activity in disintegrations per c.c. at time t = o, and N is Avogadro's number.

Reagents and apparatus. The dosimeter solution consisted of ferrous sulphate and sodium chloride both at a concentration of 10^{-3}M in 0·8N sulphuric acid. Polonium-210 was obtained as a solution in 1N nitric acid from the Radiochemical Centre, Amersham. This was then converted to nitrate-free sulphuric acid solution by thorough boiling with concentrated sulphuric acid. On one occasion a similar 0·8N sulphuric acid solution of Po-210 was supplied by Amersham on special request. The polonium was produced by irradiation of bismuth-209 and subsequent chemical separation of polonium from bismuth, and was radiochemically pure polonium-210. Analytical figures for one batch were given by Amersham as follows:

> lead and bismuth, less than 50 parts per million; iron, less than 20 parts per million; silver, less than 1 part per million; no other impurities had been identified.

Irradiations were carried out in spherical flasks ranging in volume between 10 ml and 500 ml. Since the alpha particle range in the solution was only of the order of 0·07 mm, no "wall effect", that is, significant energy loss through the walls of the containers, was expected or observed.

For the deaerated irradiations, which were carried out in 500 ml flasks only, oxygen was removed by flushing with pure moist nitrogen. For this purpose two-way stopcocks were fitted to the irradiation flasks in such a manner that the nitrogen could either bubble through the solution, or pass over the surface. After oxygen had been completely removed by bubbling nitrogen through the solution, the nitrogen stream was allowed to flow over the surface to ensure that the solutions remained oxygen-free. Polonium was introduced to the oxygen-free solutions in a special chamber, manufactured from Perspex, where the irradiation flasks could be opened in a nitrogen atmosphere (Fig. 1). Samples for spectrophotometry and radioactivity measurement were removed from these flasks by manipulation of the stopcocks and carefully increasing the nitrogen pressure on the surface so as to force out a small quantity of solution.

Adsorption. Since the polonium-210 was added to the dosimeter solution without addition of carrier material, it was necessary to establish that the polonium stayed in solution in the strong acid medium and was not significantly adsorbed on the container walls. A number of glass slides were immersed in the radioactive dosimeter solution for various times, rinsed with inactive solution, and the counting rate from both surfaces determined by liquid scintillation counting. This showed that after a few minutes an equilibrium amount of polonium was adsorbed on the glass. The amount was so small, however, that in the range of surface-to-volume ratios of the

glass apparatus used in this work, the percentage of radioactivity lost by adsorption was totally insignificant.

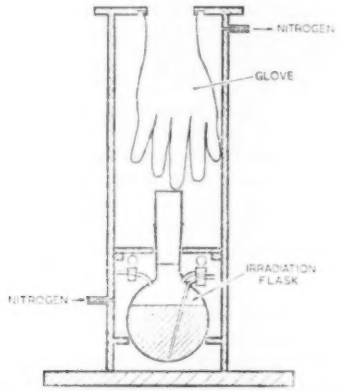

FIG. 1—Nitrogen atmosphere chamber (schematic)

Radioactivity measurements. The radioactivity of the dosimeter solutions was determined by 4π-internal liquid scintillation counting. The specific activities of these solutions ranged between 9·8 μc/gm and 25·6 μc/gm and samples taken at different times during irradiations were diluted with 2N hydrochloric acid to activity levels convenient for counting. Small aliquots of the diluted solutions were then weighed out and dissolved in 12 ml amounts of liquid scintillator solution. It was found that polonium was very efficiently carried by 2N hydrochloric acid and no significant adsorption was encountered during standardisations. The accuracy of this

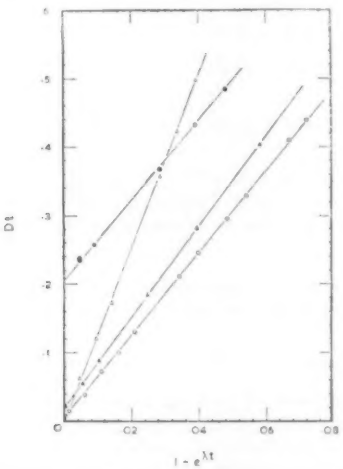

FIG.2—Linear relationship between D_t and $(1-e^{-\lambda t})$

very convenient method of α-particle counting, which was recently successfully applied to a redetermination of the U-238 half-life[9], is now well established.

RESULTS AND DISCUSSION

Altogether eight different irradiation experiments were carried out, three of which were under oxygen-free conditions. Fig. 2 shows some of the linear relationships found between Dt and $(1-e^{-\lambda t})$, the lines through the points having been calculated by the method of least squares. These lines do not pass through the origin because some of the oxidising agent H_2O_2 (produced by radiolysis) was already present in the source material *before* addition to the dosimeter solution resulting in an initial formation of ferric ions. The concentration of this H_2O_2 (and therefore of the initial ferric ions) depended on the time interval between the boiling of the polonium solution and its addition to the ferrous sulphate solution. This interval varied from about an hour to several weeks, hence the different intercepts on the t = o axis in Fig. 2.

Results for all the experiments are given in Table I where the errors listed are statistical only, calculated at the 68% confidence limit. Polonium-210 nuclear data[10] used in the calculations were: energy, 5·305 Mev.; half life, 138·4 days. No account was taken of possible effects caused by the recoiling lead-206 nucleus.

TABLE I

G-values

Aerated solutions		*Deaerated solutions*	
Experiment No.	G (ions per 100 eV.)	Experiment No.	G (ions per 100 eV.)
1	5·325 ± 0·127	1	3·534 ± 0·014
2	5·077 ± 0·029	2	3·408 ± 0·045
3	5·053 ± 0·094	3	3·528 ± 0·088
4	5·028 ± 0·089		
5	5·089 ± 0·036	Weighted mean	3·526 ± 0·013
Weighted mean	5·084 ± 0·021		

Our G-value for the aerated solution thus confirms Trumbore and Hart's value of 5·1 and disagrees with Lefort and Tarrago's result of 5·5. Our value for deaerated solutions also agrees better with that of Trumbore and Hart[7] (3·57) rather than with that of Lefort[11] (3·65).

From the currently accepted mechanism of the radiolysis of water, Barr and Schuler[12] deduced the following relationship:

$$G_{H_2} = G\,{}^{(Fe^{+++}}_{\text{deaerated}} - \tfrac{1}{2}\,G\,{}^{(Fe^{+++})}_{\text{aerated}} + \tfrac{3}{2}\Delta \quad .. \quad .. \quad .. \quad (3)$$

where G_{H_2} is the *primary* yield of hydrogen gas, which will in general be different from the experimentally determined hydrogen gas yield. Δ is a term accounting for the fact that a fraction of the hydrogen radicals which otherwise combine to form hydrogen gas is scavenged by oxygen.[12] Δ can be found from observations on the ceric-cerous system,[12] since

$$\Delta = G^{(Ce+++)}_{aerated} - G^{(Ce+++)}_{deaerated} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

A value of 0·15 for Δ is calculated from Lefort and Tarrago's data[6] for this system. (A relatively large uncertainty in this figure would not invalidate the reasoning that follows.) If this value for Δ and Trumbore and Hart's results for $G_{aerated}$ and $G_{deaerated}$ are substituted in equation (3), the primary hydrogen gas yield is obtained as:

$$G_{H_2} = 3{\cdot}57 - 2{\cdot}55 + 0{\cdot}225 = 1{\cdot}25$$

If on the other hand Lefort and Tarrago's values are substituted, G_{H_2} is found as:

$$G_{H_2} = 3{\cdot}65 - 2{\cdot}75 + 0{\cdot}225 = 1{\cdot}13$$

These two values can now be compared to hydrogen gas yields determined directly by the respective authors.

G_{H_2} should be nearly equal to the observed yield $G(H_2)$ in the aerated ferrous-ferric system or to $G(H_2)$ in the aerated as well as deaerated ceric-cerous systems. Trumbore and Hart[7] reported a value of 1·28 for $G(H_2)$ in the ferrous-ferric system containing oxygen while Lefort and Tarrago published a value of 1·57 for this yield in the cerric-cerous system. Trumbore and Hart's system contained only 0·14 mM oxygen instead of the 0·22 mM corresponding to air-saturation. In an air-saturated system the value for $G(H_2)$ would presumably have been somewhat lower. It is clear, however, that the experimental results of Trumbore and Hart, rather than those of Lefort and Tarrago, are more consistent with the predictions of theory as embodied in relationship (3).

A preliminary report of this work has been published.[13]

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REFERENCES

1 Burch, *Radiation Research*, 1959, **11**, 48.
2 Schuler and Allen, *J. Am. Chem. Soc.*, 1957, **79**, 1565.
3 Haissinsky and Anta, *Compt. rend.*, 1953, **236**, 1161.
4 Miller and Wilkenson, *Trans. Faraday Soc.*, 1954, **50**, 690.
5 McDonell and Hart, *J. Am. Chem. Soc.*, 1954, **76**, 2121.
6 Lefort and Tarrago, *J. Phys. Chem.*, 1959, **63**, 833.
7 Trumbore and Hart, *ibid.*, 1959, **63**, 867.
8 Peisach and Steyn, *J. S. African Chem. Inst.*, 1960, **13**, 34.
9 Steyn, *Proc. Symposium Metrology Radionuclides*, Vienna, I.A.E.A., 1960, 155.
10 Allen, Millett and Smith, *A.E.R.E.*—R2938, 1959.
11 Lefort, *Compt. rend.*, 1957, **245**, 1625.
12 Barr and Schuler, *J. Phys. Chem.*, 1959, **63**, 808.
13 Stein and van As, *Nature*, 1961, **191**, 903.

NOTES

SOME SOURCES OF ERROR IN THE MEASUREMENT OF SOIL pH VALUES

by

J. N. S. HILL

It has often been observed during routine laboratory work that the pH values of soil samples kept for more than a week could not be satisfactorily duplicated and that different glass electrodes gave different results. The following factors might be contributory: cleanliness of the glass electrodes, rate of stirring while taking readings, duration of shaking while preparing suspensions, and finally, nature of the junction between KCl solution and the soil suspension in the calomel or reference electrode.

To study this problem, twenty widely varying Natal soils were selected, and suspensions prepared according to the recommendations of the Soil Reaction Committee of the International Society of Soil Science, namely by shaking 1 part of soil with 2·5 parts of water.

Three different models of pH meters were employed, each being equipped with a standard glass electrode, and the following aspects examined:

Cleanliness of the glass electrodes and the effect of stirring whilst reading pH values. Ten of the twenty soils, randomly selected, were tested on two meters using the same reference electrode in each case but using three glass electrodes in succession. One of the glass electrodes had a dirty membrane, another had only a slightly dirty membrane and the third had a very clean membrane. The following table shows the results obtained. It was noticeable that the dirtier the glass electrode the less effect stirring had on the pH reading.

TABLE I

Effect of mechanical stirring and of cleanliness of the glass electrode on the pH values of selected Natal soils

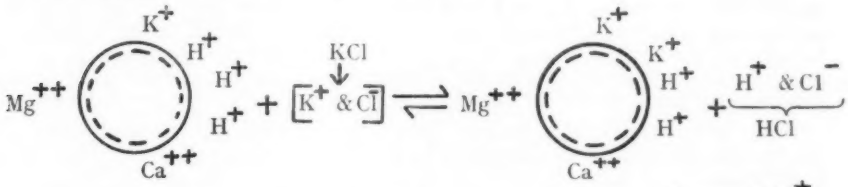
	Glass electrode					
	Dirty		Partly dirty		Clean	
Soil	Stirring	not stirring	Stirring	not stirring	stirring	not stirring
1	5·75	5·80	5·75	5·80	5·50	5·80
2	6·15	6·20	5·90	5·95	5·60	5·90
3	6·00	6·00	5·95	6·00	5·80	6·00
4	6·40	6·45	6·35	6·45	6·25	6·40
5	6·25	6·30	6·10	6·25	5·90	6·25
6	5·60	5·60	5·50	5·60	5·30	5·60
7	5·10	5·15	5·15	5·20	5·00	5·20
8	5·00	5·05	5·05	5·10	4·95	5·10
9	5·70	5·70	5·60	5·70	5·55	5·75
10	8·50	8·50	8·55	8·60	8·45	8·60
Mean difference		+0·03		+0·08		+0·23

Duration of shaking on the preparation of soil suspensions. The twenty soil solution suspensions were each prepared by shaking for one minute[1] and their pH values read immediately, mechanical stirring being employed. The suspensions were then mechanically shaken on an end-to-end shaker for one hour and the pH values again

determined. The readings obrained were similar to the first series, indicating that as far as Natal coastal soils were concerned equilibrium was rapidly attained[1] with the water, and thus for our purposes, the "one-minute" techique is quite suitable. The effect of the quality of the distilled water used was also examined and it was found that stabilisation of the CO_2-content of the water made no difference in readings as far as soil pH values were concerned, but this may be the case only with acid Natal coastal soils.

Effect of KCl seepage from the reference electrode. If one reference electrode allows more seepage of KCl through the junction than another during the pH measurement of the soil suspension, then a lower pH will be recorded for the electrode with the greater seepage, i.e., exchange of cations takes place, H^+ being replaced by K^+ with consequent drop in pH.

The suggested reactions are represented below:—



This effect of seepage is supported by the following experiment with a new "combination" electrode, in which the glass half cell was surrounded by the KCl solution of the reference half cell. It was found that this electrode with its wick and consequently greated KCl seepage caused exchange to take place to such an extent that the pH meter needle could actually be seen drifting lower whilst the reading was being taken. Distilled water and buffer solutions were not affected by this seepage owing to lack of colloidal material for the exchange reaction, whilst strongly buffeted soils also did not show much change, simply because any effect was masked by the buffering action. However, weakly buffered sandy soils showed a fairly rapid drift in the short time taken to read the pH.

Of a series of pH meters used, three standard models were selected for study and a large number of replications carried out with fresh suspensions in each case. Two of the models were mains operated and one a battery set.

The results of this work showed that in general replicability, when using scrupulously clean glass electrodes and mechanically stirring while reading, was excellent. Uniform stirring was affected by mounting the beaker on a platform driven by a gramophone motor. Under these conditions there was no variation in reading between the different types of pH meter used.

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REFERENCE

[1] Piper, *Soil and Plant Analysis*, Hassell Press, Adelaide, Australia, 1942, pp. 7-9.

QUALITATIVE ANALYSIS OF URANIUM SOLUTIONS BY WEISZ RING OVEN TECHNIQUE

by

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Qualitative analysis of uranium solutions has been carried out by the Weisz Ring Oven Method,[1] coupled with separation of metals into groups by means of solvent extraction and identification of individual ions by the spot test techniques of West and Mukherji.[2]

Briefly the method is as follows: A few drops of nitric acid are added to 3 ml of the solution under test and the solution is evaporated to dryness. The residue is dissolved in 1 ml 7M hydrochloric acid and silica is filtered off. The solution is then successively extracted, after addition of appropriate reagents, by different solvents. In this way the ions present in the solution are separated into groups. Part of each group extract is washed into the ring by the Weisz method and the individual ions identified by spot tests.

The above technique of analysis proved to be simple and convenient and had considerable sensitivity. Metals present in near trace quantities in the solution were easily detected. It was possible to analyse four different solutions for 28 elements during a working day.

Barren solutions from seventeen uranium-producing mines were analysed in this way. It was found that in all solutions Mo, Fe, As, Sb, Co, Zn, Sn, Al, Cu, Cr, Cd, Mn, Ni, Ca and Mg were present. Seven solutions contained Zr, three Be, three Ti, and one Ce. Ga, Ge, V, Hg, Bi, Se, Tl, W and Pb were not detected in any of the solutions.

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Chamber of Mines Biological and Chemical Research Laboratory,
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Johannesburg.

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REFERENCES

[1] Weisz, "The Ring Oven Method," in Wilson and Wilson, *Comprehensive Analytical Chemistry*, Elsevier, Amsterdam, 1959, p. 369.
[2] West and Mukherji, *Anal. Chem.*, 1959, **31**, 947.

RETRORSINE FROM *CROTALARIA SPARTIOIDES*

by

S. W. D. BRÜMMERHOFF and H. L. DE WAAL

A broomy shrub, identified as *Crotalaria spartioides* („Duinebos," „Besembos") and collected in the mature stage in the Kalihari Gemsbok Park, was found to contain the physiologically-active alkaloid retrorsine, a well-known alkaloid of several spp. of *Senecio*. As far as could be ascertained from the literature this is the first time that retrorsine has been isolated from a *Crotalaria* species. However, *Senecio* and *Crotalaria* alkaloids are chemically closely related and cause similar cymptoms of liver cirrhosis. The alkaloid was present in a yield of 0·05% in the dried plant material. Its properties as well as the properties of its fission products were identical with those of authentic retrorsine from *S. retrorsus* D.C. and the latter's fission products respectively. No other alkaloids could be detected in the plant.

EXPERIMENTAL

Extraction. 1·4 Kg dried and ground *Crotalaria spartioides* was extracted (i) with 6 litres 96% ethanol at room temperature followed (ii) by 6 litres 96% ethanol on a boiling waterbath. From both filtrates worked up separately, after the evaporation of the alcohol, acidification with citric acid solution and extraction of alkaloids in the usual way[1] only retrorsine could be detected and was isolated in a total yield of 0·75 g. Its solubility, crystallisability, m.p., reaction towards alkaloidal reagents and Rf value (see below) were identical with that of authentic retrorsine[1]. A mixed m.p. gave no depression. (Found: C, 61·8; H, 7·2; N, 3·6. Calc. for $C_{18}H_{25}O_6N$: C, 61·5; H, 7·2; N, 4·0%).

Hydrolysis. The alkaloid (300 mg) in ethanol (15 ml) was refluxed with KOH (0·12g) for one hour. The isolation of the base, retronecine, and the acid, retronecic acid, was effected as previously described[2]. Melting points were identical and mixed m.p.'s gave no depression with authentic samples of retronecine and retronecic acid respectively.

Chromatography. A most convenient method for the identification of *Senecio* alkaloids by paper chromatography as well as the determination of their Rf values is as follows. The descending Whatman No. I paper chromatogram in a mixture of butanol-acetic acid-water (100:20:46) gives for each of the following alkaloids one distinct spot after development of the chromatogram with a solution of bismuth subnitrate and potassium iodide in dilute acetic acid.[3] The Rf values thus obtained for retrorsine and sceleratine are 0·66 and 0·59 respectively.

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REFERENCES

[1] de Waal, *Onderstepoort J. Vet. Research*, 1939, **12**, 155; de Waal and Pretorius, *ibid.*, 1941, **17**, 181.
[2] de Waal, *ibid.*, 1941, **16**, 163.
[3] Munier and Macheboeuf, *Bull. Soc. Chim. Biol.*, 1951, **33**, 846; Schöpf and de Waal, *Chem. Ber.*, 1956, **89**, 922.

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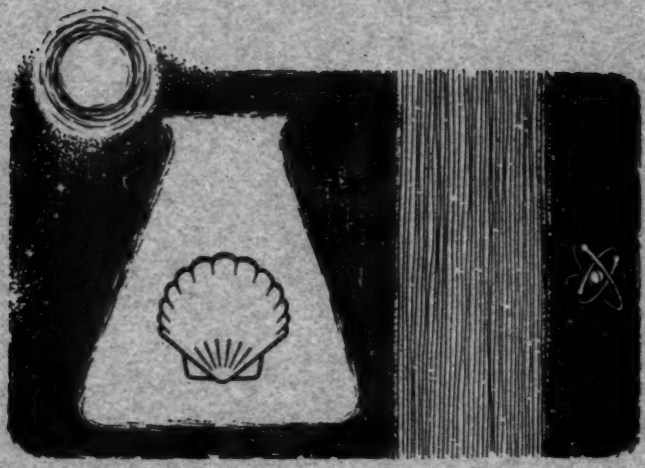

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